fusions between catala[redacted]A and human catalase which inc[redacted]de the catalase A internal PT[redacted]are **targeted**, at least in part, [redacted] **peroxisomes** regardless of whether the COOH-terminal human catalase PTS is intact.

1996

13/3,AB/18 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10462952 BIOSIS NO.: 199699084097
Labeling of **peroxisomes** with green fluorescent **protein** in living P. pastoris cells.
AUTHOR: Monosov Edward Z; Wenzel Thibaut J; Luers Georg H; Heyman John A; Subramani Suresh(a)
AUTHOR ADDRESS: (a)Dep. Biol., Rm 4314 Bonner Hall, Univ. California, La Jolla, CA 92093-0322**USA
JOURNAL: Journal of Histochemistry and Cytochemistry 44 (6):p581-589 1996
ISSN: 0022-1554
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We exploited the light-activated fluorescent properties of the green fluorescent **protein** (GFP) of the jellyfish Aequorea victoria for studies on the **peroxisomal** sorting of polypeptides. GFP and GFP-SKL (containing a C-terminal, tripeptide **peroxisomal targeting** signal, SKL) were **expressed** from a methanol-inducible, alcohol oxidase (AOX1) promoter in the methylotrophic yeast Pichia pastoris. GFP was cytosolic, whereas the GFP-SKL **fusion protein** was **targeted** to **peroxisomes**, as demonstrated by biochemical fractionation of organelles on Nycodenz gradients. Neither GFP nor GFP-SKL affected the viability of yeast cells but both were fluorescent on excitation with 395-nm uv light. The subcellular locations of GFP and GFP-SKL in living yeast cells were monitored by fluorescence microscopy and their fluorescence was coupled to photo-oxidation of diaminobenzidine (DAB), resulting in the deposition of electron-dense oxidized DAB at intracellular locations of GFP derivatives. This photooxidation procedure permitted facile ultrastructural localization of GFP in cells by electron microscopy, and provided further evidence that GFP produced in P. pastoris is cytosolic, whereas GFP-SKL is **peroxisomal**. The GFP-SKL **fusion protein** is therefore a versatile reporter for the **peroxisomal** compartment, with many applications for studies involving **peroxisomal** import and biogenesis.

1996

13/3,AB/19 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10269365 BIOSIS NO.: 199698724283
Peb1p (Pas7p) is an intraperoxisomal receptor for the NH-1-terminal, type 2, **peroxisomal targeting** sequence of thiolase: Peb1p itself is **targeted** to **peroxisomes** by an NH-2-terminal peptide.
AUTHOR: Zhang Jing Wei(a); Lazarow Paul B
AUTHOR ADDRESS: (a)Dep. Cell Biol., Anatomy, Box 1007, Mount Sinai Sch. Med., One Gustave Levy Place, New York, NY **USA
JOURNAL: Journal of Cell Biology 132 (3):p325-334 1996
ISSN: 0021-9525
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Peb1 is a **peroxisome** biogenesis mutant isolated in Saccharomyces cerevisiae that is selectively defective in the import of thiolase into **peroxisomes** but has a normal ability to package catalase, luciferase and acyl-CoA oxidase (Zhang, J. W., C. Luckey, and P. B. Lazarow. 1993. Mol. Biol. Cell. 4:1351-1359). Thiolase differs from these other **peroxisomal proteins** in that it is **targeted** by an NH-2-terminal, 16-amino acid **peroxisomal targeting** sequence type 2 (PTS 2). This phenotype suggests that the PEB1 **protein** might function as a receptor for the PTS2. The PEB1 gene has been cloned by functional complementation. It encodes a 42,320-D, hydrophilic **protein** with no predicted transmembrane segment. It contains six WD repeats that comprise the entire **protein** except for the first 55 amino acids. Peb1p was tagged with hemagglutinin epitopes and determined to be exclusively within **peroxisomes** by digitonin permeabilization, immunofluorescence, protease protection and immuno-electron microscopy (Zhang, J. W., and P. B. Lazarow. 1995. J. Cell. Biol. 129:65-80). Peb1p is identical to Pas7p (Marzioch, M., R. Erdmann, M. Veenhuis, and W.-H. Kunau. 1994. EMBO J. 13:4908-4917). We now have tested whether Peb1p interacts with the PTS2 of thiolase. With the two-hybrid assay, we observed a strong interaction between Peb1p and thiolase that was abolished by deleting the first 16 amino acids of thiolase. An oligopeptide consisting of the first 16 amino acids of thiolase was sufficient for the affinity binding of Peb1p. Binding was reduced by the replacement of leucine with arginine at residue five, a change that is known to reduce thiolase **targeting** in vivo. Finally, a thiolase-Peb1p complex was isolated by immunoprecipitation. To investigate the topogenesis of Peb1p, its first 56-amino acid residues were fused in front of truncated thiolase lacking the NH-2-terminal 16-amino acid PTS2. The **fusion protein** was **expressed** in a thiolase knockout strain. Equilibrium density centrifugation and immunofluorescence indicated that the **fusion protein** was located in **peroxisomes**. Deletion of residues 6-55 from native Peb1p resulted in a cytosolic location and the loss of function. Thus the NH-2-terminal 56-amino acid residues of Peb1p are necessary and sufficient for **peroxisomal targeting**. Peb1p is found in **peroxisomes** whether thiolase is **expressed** or not. These results suggest that Peb1p (Pas7p) is an intraperoxisomal receptor for the type 2 **peroxisomal targeting** signal.

1996

13/3,AB/20 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09921139 BIOSIS NO.: 199598376057
Human **Peroxisomal Targeting** Signal-1 Receptor Restores **Peroxisomal Protein** Impact in Cells from Patients with Fatal **Peroxisomal** Disorders.
AUTHOR: Wiemer Erik A C; Nuttley William M; Bertolaet Bonnie L; Li Xu; Francke Uta; Wheelock Margaret J; Anne Usha K; Johnson Keith R; Subramani Suresh(a)
AUTHOR ADDRESS: (a)Dep. Biol., Bonner Hall, Univ. California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0322**USA
JOURNAL: Journal of Cell Biology 130 (1):p51-65 1995
ISSN: 0021-9525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Two **peroxisomal targeting** signals, PTS1 and PTS2, are

involved in the import of **proteins** into the **peroxisome** matrix. Human patients with fatal generalized **peroxisome** deficiency disorders fall into at least nine genetic complementation groups. Cells from many of these patients are deficient in the import of PTS1-containing **proteins**, but the causes of the **protein** -import defect in these patients are unknown. We have cloned and sequenced the human cDNA homologue (PTS1R) of the Pichia pastoris PAS8 gene, the PTS1 receptor (McCollum, D., E. Monosov, and S. Subramani. 1993. J. Cell Biol. 121:761-774). The PTS1R mRNA is **expressed** in all human tissues examined. Antibodies to the human PTS1R recognize this **protein** in human, monkey, rat, and hamster cells. The **protein** is localized mainly in the cytosol but is also found to be associated with **peroxisomes**. Part of the **peroxisomal** PTS1R **protein** is tightly bound to the **peroxisomal** membrane. Antibodies to PTS1R inhibit **peroxisomal protein**-import of PTS1-containing **proteins** in a permeabilized CHO cell system. In vitro-translated PTS1R **protein** specifically binds a serine-lysine-leucine-peptide. A PAS8-PTS1R **fusion protein** complements the P. pastoris pas8 mutant. The PTS1R cDNA also complements the PTS1 **protein**-import defect in skin fibroblasts from patients-belonging to complementation group two-diagnosed as having neonatal adrenoleukodystrophy or Zellweger syndrome. The PTS1R gene has been localized to a chromosomal location where no other **peroxisomal** disorder genes are known to map. Our findings represent the only case in which the molecular basis of the **protein**-import deficiency in human **peroxisomal** disorders is understood.

1995

13/3,AB/21 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07319408 BIOSIS NO.: 000090099308
FATE OF HIGHLY **EXPRESSED PROTEINS** DESTINED TO **PEROXISOMES** IN SACCHAROMYCES-CEREVISIAE
AUTHOR: HARTIG A; OGRIS M; COHEN G; BINDER M
AUTHOR ADDRESS: INST. ALLGEMEINE BIOCHEMIE, UNIV. WIEN, WAEHRINGER STR. 38, A-1090 WIEN, AUSTRIA.
JOURNAL: CURR GENET 18 (1). 1990. 23-28. 1990
FULL JOURNAL NAME: Current Genetics
CODEN: CUGED
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Import of **proteins** into organelles usually requires a cis-acting **targeting** signal. Analysis of various hybrid **proteins**, consisting of mouse DHFR and parts of catalase A from Saccharomyces cerevisiae, revealed that **fusion proteins** containing the N-terminal 126 amino acids, or less, of catalase A remain in the cytosol whereas **fusion proteins** containing 140, or more, N-terminal amino acids of catalase A form large aggregates inside the cell. These **protein** bodies, which lack a surrounding membrane, copurified with **peroxisomes** on cell fractionation. The **peroxisomal targeting** signal of catalase A does not reside at the C-terminus or at the N-terminus.

1990

13/3,AB/22 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06603629 BIOSIS NO.: 000087045791

ACYL COENZYME A OXIDASE CONTAINS TWO **TARGETING** SEQUENCES EACH OF WHICH CAN MEDIATE **PROTEIN** IMPORT INTO **PEROXISOMES**
AUTHOR: SMALL G M; SZABO L J; LAZAROW P B
AUTHOR ADDRESS: ROCKEFELLER UNIV., 1230 YORK AVE., NEW YORK, N.Y. 10021.
JOURNAL: EMBO (EUR MOL BIOL ORGAN) J 7 (4). 1988. 1167-1174. 1988
FULL JOURNAL NAME: EMBO (European Molecular Biology Organization) Journal
CODEN: EMJOD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Acyl-CoA oxidase is a major induced enyzme in **peroxisomes** of Candida tropicalis grown on fatty acids. The gene, POX4, encoding acyl-CoA oxidase was **expressed** in vitro, and the resulting polypeptide was imported into purified **peroxisomes** in a temperature-dependent fashion. Plasmids containing fragments of POX4 were prepared, **expressed** and the polypeptides tested for import into **peroxisomes**. We identified two regions of acyl-CoA oxidase (amino acids 1-118 and 309-427) contained information that specifically **targeted** fragments of acyl-CoA oxidase to **peroxisomes**. The corresponding regions of the gene were fused to cDNA encoding the cytosolic enzyme dihydrofolate reductase (DHFR), and the **expressed fusion proteins** were likewise imported into **peroxisomes** . DHFR itself neither bound to, nor was important into **peroxisomes**. Thus, there are at least two regions of **peroxisomal targeting** information in the acyl-CoA oxidase gene.

1988

13/3,AB/23 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3502845 20508739 Holding Library: AGL
Peb1p(Pas7p) is an intraperoxisomal receptor for the NH2-ternal, type2, **peroxisomal targeting** sequence of thiolase: Peb1p itself is **targeted** to **peroxisomes** by an NH2-terminal peptide
Zhang, J.W. Lazarow, P.B.
Mount Sinai School of Medicine, New York, NY.
New York : Rockefeller University Press, 1962-
The Journal of cell biology. Feb 1996. v. 132 (3) p. 325-334.
ISSN: 0021-9525 CODEN: JCLBA3
DNAL CALL NO: 442.8 J828
Language: English
Peb1 is a **peroxisome** biogenesis mutant isolated in Saccharomyces cerevisiae that is selectively defective in the import of thiolase into **peroxisomes** but has a normal ability to package catalase, luciferase and acyl-CoA oxidase (Zhang, J. W., C. Luckey, and P. B. Lazarow. 1993. Mol. BioL Cell. 4:1351-1359). Thiolase differs from these other **peroxisomal proteins** in that it is **targeted** by an NH2-terminal, 16-amino acid **peroxisomal targeting** sequence type 2 (PTS 2). This phenotype suggests that the PEB1 **protein** might function as a receptor for the PTS2. The PEB1 gene has been cloned by functional complementation. It encodes a 42,320-D, hydrophilic **protein** with no predicted transmembrane segment. It contains six WD repeats that comprise the entire **protein** except for the first 55 amino acids. Peb1p was tagged with hemagglutinin epitopes and determined to be exclusively within **peroxisomes** by digitonin permeabilization, immunofluorescence, protease protection and immuno-electron microscopy (Zhang, J. W., and P.B. Lazarow. 1995. J. Cell Biol. 129:65-80). Peb1p is identical to Pas7p (Marzioch, M., R. Erdmann, M. Veenhuis, and W.-H. Kunau. 1994. EMBO J. 13: 4908-4917). We have now tested whether Peb1p interacts with the PTS2 of thiolase. With the two-hybrid as-say, we observed a strong interaction between Peb1p and thiolase that was abolished by deleting the first 16 amino acids of thiolase. An oligopeptide consisting of the first

16 amino acids of thiolase was sufficient for the affinity binding of Peb1p. Binding was reduced by the replacement of leucine with arginine at residue five, a change that is known to reduce thiolase **targeting** in vivo. Finally, a thiolase-Peb1p complex was isolated by immunoprecipitation. To investigate the topogenesis of Peb1p, its first 56-amino acid residues were fused in front of truncated thiolase lacking the NH2-terminal 16-amino acid PTS2. The **fusion protein** was **expressed** in a thiolase knockout strain. Equilibrium density centrifugation and immunofluorescence indicated that the **fusion protein** was located in **peroxisomes**. Deletion of residues 6-55 from native Peb1p resulted in a cytosolic location and the loss of function. Thus the NH2-terminal 56-amino acid residues of Peb1p are necessary and sufficient for **peroxisomal targeting**. Peb1p is found in **peroxisomes** whether thiolase is **expressed** or not. These results suggest that Peb1p (Pas7p) is an intraperoxisomal receptor for the type 2 **peroxisomal targeting** signal.

13/3,AB/24 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08233021 Genuine Article#: 260YF Number of References: 96
Title: **Peroxisomal** membrane ascorbate peroxidase is sorted to a membranous network that resembles a subdomain of the endoplasmic reticulum (ABSTRACT AVAILABLE)
Author(s): Mullen RT; Lisenbee CS; Miernyk JA; Trelease RN (REPRINT)
Corporate Source: ARIZONA STATE UNIV,DEPT PLANT BIOL/TEMPE//AZ/85287 (REPRINT); ARIZONA STATE UNIV,DEPT PLANT BIOL/TEMPE//AZ/85287; ARIZONA STATE UNIV,GRAD PROGRAM MOL & CELLULAR BIOL/TEMPE//AZ/85287; USDA ARS,NATL CTR AGR UTILIZAT RES/PEORIA//IL/61604
Journal: PLANT CELL, 1999, V11, N11 (NOV), P2167-2185
ISSN: 1040-4651 Publication date: 19991100
Publisher: AMER SOC PLANT PHYSIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855
Language: English Document Type: ARTICLE
Abstract: The **peroxisomal** isoform of ascorbate peroxidase (APX) is a novel membrane isoform that functions in the regeneration of NAD(+) and protection against toxic reactive oxygen species. The intracellular localization and sorting of **peroxisomal** APX were examined both in vivo and in vitro. Epitope-tagged **peroxisomal** APX, which was **expressed** transiently in tobacco BY-2 cells, localized to a reticular/circular network that resembled endoplasmic reticulum (ER; 3,3'-dihexyloxa-carbocyanine iodide-stained membranes) and to **peroxisomes**. The reticular network did not colocalize with other organelle marker **proteins**, including three ER reticuloplasmins, However, in vitro, **peroxisomal** APX inserted posttranslationally into the ER but not into other purified organelle membranes (including **peroxisomal** membranes). Insertion into the ER depended on the presence of molecular chaperones and ATP, These results suggest that regions of the ER serve as a possible intermediate in the sorting pathway of **peroxisomal** APX. Insight into this hypothesis was obtained from in vivo experiments with brefeldin A (BFA), a toxin that blocks vesicle-mediated **protein** export from ER, A transiently **expressed** chloramphenicol acetyltransferase-**peroxisomal** APX (CAT-pAPX) **fusion protein** accumulated only in the reticular/circular network in BFA-treated cells; after subsequent removal of BFA from these cells, the CAT-pAPX was distributed to preexisting **peroxisomes**. Thus, **plant peroxisomal** APX, a representative enzymatic **peroxisomal** membrane **protein**, is sorted to **peroxisomes** through an indirect pathway involving a preperoxisomal compartment with characteristics of a distinct subdomain of the ER, possibly a **peroxisomal** ER subdomain.

13/3,AB/25 (Item 2 [redacted]om file: 34)
DIALOG(R)File 34:SciSea[redacted](R) Cited Ref Sci

07316582 Genuine Article#: 149VA Number of References: 45
Title: Characterization of intermediates in the process of **plant peroxisomal protein** import (ABSTRACT AVAILABLE)
Author(s): Pool MR; LopezHuertas E; Baker A (REPRINT)
Corporate Source: UNIV LEEDS,INST PLANT BIOTECHNOL & AGR, CTR PLANT SCI/LEEDS LS2 9JT/W YORKSHIRE/ENGLAND/ (REPRINT); UNIV LEEDS,INST PLANT BIOTECHNOL & AGR, CTR PLANT SCI/LEEDS LS2 9JT/W YORKSHIRE/ENGLAND/
Journal: EMBO JOURNAL, 1998, V17, N23 (DEC 1), P6854-6862
ISSN: 0261-4189 Publication date: 19981201
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND
Language: English Document Type: ARTICLE
Abstract: A hybrid **protein** in which the immunoglobulin G-binding domain of Staphylococcus aureus **protein** A replaced the N-terminal 43 amino acids of glycolate oxidase (a **peroxisomal protein**) was affinity purified after **expression** in Escherichia coli and used to study **peroxisomal protein** import in vitro. The **fusion protein**, which co-purifies with the bacterial chaperones dnaK and groEL, binds to glyoxysomes and is partially translocated in an ATP-dependent reaction which is independent of eukaryotic cytosol. Both binding and translocation are dependent upon the amount of glyoxysomes present. The partially translocated species has a transmembrane location and is extractable by salt, indicating that it is held in the membrane by ionic interactions. In the absence of ATP, the **fusion protein** binds to the surface of the glyoxysomes and competes the binding of authentic matrix **proteins** . The surface-bound **protein** can be chased to the transmembrane species upon the addition of ATP, These results indicate that the surface-bound form is a true translocation intermediate. The availability of this **fusion protein** in milligram quantities offers the possibility to use the intermediate formed in the absence of ATP and the transmembrane species to probe interactions with the **peroxisome** import machinery.

13/3,AB/26 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06726618 Genuine Article#: ZN588 Number of References: 49
Title: Engineering in vivo instability of firefly luciferase and Escherichia coli beta-glucuronidase in higher **plants** using recognition elements from the ubiquitin pathway (ABSTRACT AVAILABLE)
Author(s): Worley CK; Ling R; Callis J (REPRINT)
Corporate Source: UNIV CALIF DAVIS,SECT MOL & CELLULAR BIOL, 1 SHIELDS AVE/DAVIS//CA/95616 (REPRINT); UNIV CALIF DAVIS,SECT MOL & CELLULAR BIOL/DAVIS//CA/95616
Journal: PLANT MOLECULAR BIOLOGY, 1998, V37, N2 (MAY), P337-347
ISSN: 0167-4412 Publication date: 19980500
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: The ubiquitin pathway **targets proteins** for degradation through the post-translational covalent attachment of the 76 amino acid **protein** ubiquitin to epsilon-amino lysyl groups on substrate **proteins**. Two instability determinants recognized by the ubiquitin pathway in Saccharomyces cerevisiae have been identified. One is described by the N-end rule and requires specific destabilizing residues at the substrate **protein** N-termini along with a proximal lysyl residue for ubiquitin conjugation. The second is a linear uncleavable N-terminal ubiquitin moiety. The ability of these two determinants to function in higher **plants** was investigated in

tobacco protoplast sient transfection assays using DNA encoding variants of well ch terized reporter enzymes as s[redacted]trates: firefly luciferase that is localized to **peroxisomes** (pxLUC), a cytosolic version of LUC (cLUC), and Escherichia coli beta-glucuronidase (GUS). cLUC with phenylalanine encoded at its mature N-terminus was 10-fold less abundant than cLUC with methionine at its mature N-terminus. GUS with phenylalanine encoded at its mature N-terminus was 3-fold less abundant than GUS with methionine at its mature N-terminus. The presence of a uncleavable N-terminal ubiquitin **fusion** resulted in 50-fold lower **protein** accumulation of cLUC, but had no effect on GUS. Both instability determinants had a much larger effect on cLUC than on pxLUC, suggesting that these degradation signals are either unrecognized or poorly recognized in the **peroxisomes**.

13/3,AB/27 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06284466 Genuine Article#: YF800 Number of References: 45
Title: Diverse amino acid residues function within the type 1 **peroxisomal targeting** signal. Implications for the role of accessory residues upstream of the type 1 **peroxisomal targeting** signal (ABSTRACT AVAILABLE)
Author(s): Mullen RT; Lee MS; Flynn CR; Trelease RN (REPRINT)
Corporate Source: ARIZONA STATE UNIV,DEPT PLANT BIOL/TEMPE//AZ/85287 (REPRINT); ARIZONA STATE UNIV,DEPT PLANT BIOL/TEMPE//AZ/85287; ARIZONA STATE UNIV,GRAD PROGRAM MOL & CELLULAR BIOL/TEMPE//AZ/85287
Journal: PLANT PHYSIOLOGY, 1997, V115, N3 (NOV), P881-889
ISSN: 0032-0889 Publication date: 19971100
Publisher: AMER SOC PLANT PHYSIOLOGISTS, 15501 MONONA DRIVE, ROCKVILLE, MD 20855
Language: English Document Type: ARTICLE
Abstract: The purpose of this study was to determine whether the **plant** type 1 **peroxisomal targeting** signal (PTS1) utilizes amino acid residues that do not strictly adhere to the serine-lysine-leucine (SKL) motif (small-basic-hydrophobic residues). Selected residues were appended to the C terminus of chloramphenicol acetyltransferase (CAT) and were tested for their ability to **target** CAT **fusion proteins** to glyoxysomes in tobacco (Nicotiana tabacum L.) cv Bright Yellow 2 suspension-cultured cells. CAT was redirected from the cytosol into glyoxysomes by a wide range of residues, i.e. A/C/G/S/T-H/K/L/N/R-I/L/M/Y. Although L and N at the -2 position (-SLL, -ANL) do not conform to the SKL motif, both functioned, but in a temporally less-efficient manner. Other SKL divergent residues, however, did not **target** CAT to glyoxysomes, i.e. F or P at the -3 position (-FKL, -PKL), S or T at the -2 position (-SSI, STL), or D at the -1 position (-SKD). The **targeting** inefficiency of CAT-ANL could be ameliorated when K was included at the -4 position (-KANL). In summary, the **plant** PTS1 mostly conforms to the SKL motif. For those PTS1s that possess nonconforming residue(s), other residues upstream of the PTS1 appear to function as accessory sequences that enhance the temporal efficiency of **peroxisomal targeting**.

13/3,AB/28 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02184575 Genuine Article#: KH290 Number of References: 31
Title: THE C-TERMINAL TRIPEPTIDE OF GLYCOSOMAL PHOSPHOGLYCERATE KINASE IS BOTH NECESSARY AND SUFFICIENT FOR IMPORT INTO THE GLYCOSOMES OF TRYPANOSOMA-BRUCEI (Abstract Available)
Author(s): SOMMER JM; PETERSON G; KELLER GA; PARSONS M; WANG CC

Corporate Source: UNIV F SAN FRANCISCO,DEPT PHARMACEUT CHEM/SAN FRANCISCO//CA/94143
Journal: FEBS LETTERS, 1993, V316, N1 (JAN 18), P53-58
ISSN: 0014-5793
Language: ENGLISH Document Type: ARTICLE
Abstract: Glycosomal phosphoglycerate kinase (gPGK) of Trypanosoma brucei differs from the cytoplasmic isozyme (cPGK) in its higher isoelectric point characterized by clusters of positive charges along the polypeptide chain, and a 20 amino acid C-terminal extension ending in serine-serine-leucine (SSL). While a C-terminal SSL tripeptide is apparently not capable of directing luciferase to the **peroxisomes** in mammalian cells [J. Cell Biol. 108 (1989),1657-1664], we show here that it is sufficient for the import of luciferase as well as an unrelated **protein**, beta-glucuronidase, into the glycosomes of T brucei, as determined by immunoelectron microscopy. The analysis of luciferase-gPGK **fusion proteins** indicates that the only **targeting** signal for import of gPGK into the glycosome resides in this C-terminal SSL sequence.

13/3,AB/29 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

03939132 CAB Accession Number: 20001614419
Importance of sequences adjacent to the terminal tripeptide in the import of a **peroxisomal** Candida tropicalis **protein** in **plant peroxisomes**.
Bongcam, V.; Petetot, J. M. C.; Mittendorf, V.; Robertson, E. J.; Leech, R. M.; Qin YongMei; Hiltunen, J. K.; Poirier, Y.
Institut d'Ecologie-Biologie et Physiologie Vegetales, Universite de Lausanne, 1015 Lausanne, Switzerland.
Planta vol. 211 (1): p.150-157
Publication Year: 2000
ISSN: 0032-0935 --
Language: English
Document Type: Journal article
The **peroxisome targeting** signal (PTS) required for import of the rat acyl-CoA oxidase (AOX; EC 1.3.3.6) and the Candida tropicalis multifunctional **protein** (MFP) in **plant peroxisomes** was assessed in transgenic Arabidopsis thaliana. The native rat AOX accumulated in **peroxisomes** in A. thaliana cotyledons and **targeting** was dependent on the presence of the C-terminal tripeptide S-K-L. In contrast, the native C. tropicalis MFP, containing the consensus PTS sequence A-K-I was not **targeted** to **plant peroxisomes**. Modification of the carboxy terminus to the S-K-L tripeptide also failed to deliver the MFP to **peroxisomes** while addition of the last 34 amino acids of the Brassica napus isocitrate lyase, containing the terminal tripeptide S-R-M, enabled import of the **fusion protein** into **peroxisomes**. These results underline the influence of the amino acids adjacent to the terminal tripeptide of the C. tropicalis MFP on **peroxisomal targeting**, even in the context of a **protein** having a consensus PTS sequence S-K-L. 48 ref.

13/3,AB/30 (Item 2 from file: 50)
DIALOG(R)File 50:CAB Abstracts

02814806 CAB Accession Number: 942300993
Targeting of glyoxysomal **proteins** to **peroxisomes** in leaves and roots of a higher **plant**.
Olsen, L. J.; Ettinger, W. F.; Damsz, B.; Matsudaira, K.; Webb, M. A.; Harada, J. J.
Section of Botany, Division of Biological Sciences, University of

California, Davis, CA .6, USA.
Plant Cell vol. 5 (p.941-952
Publication Year: 1993
ISSN: 1040-4651 --
Language: English
Document Type: Journal article

Higher **plants** possess several classes of **peroxisomes** that are present at distinct developmental stages and serve different metabolic roles. In order to investigate the cellular processes that regulate developmental transitions of **peroxisomal** function, the **targeting** of glyoxysomal **proteins** to leaf-type and root **peroxisomes** was analysed. Cell fractionation and immunogold localization experiments revealed that when genes encoding the glyoxysome-specific enzymes isocitrate lyase (IL) and malate synthase were transferred into Arabidopsis thaliana **plants**, the glyoxysomal **proteins** were imported into leaf-type and root **peroxisomes**. The sequences that **target** IL to **peroxisomes** were defined and the question as to whether the same **targeting** determinant is recognized by different classes of the organelle was investigated. By localizing deletion and **fusion** derivatives of IL, it was shown that the polypeptide's carboxyl terminus is both necessary for its transport to **peroxisomes** and sufficient to redirect a passenger **protein** from the cytosol to both glyoxysomes and leaf-type **peroxisomes**. Thus, it was concluded that glyoxysomal **proteins** are transported into several classes of **peroxisomes** using a common **targeting** determinant, so suggesting that **protein** import does not play a regulatory role in determining a **peroxisome**'s function. Rather, the specific metabolic role of a **peroxisome** appears to be determined primarily by processes that regulate the synthesis and/or stability of its constituent **proteins** . These processes are specified by the differentiated state of the cells in which the organelles are found. 55 ref.

13/3,AB/31 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02172638 4094461
Transport of chimeric **proteins** that contain a carboxyterminal **targeting** signal into **plant** microbodies
Hayashi, M.; Aoki, M.; Kato, A.; Kondo, M.; Nishimura, M.
Department of Cell Biology, National Institute for Basic Biology, Okazaki 444, Japan
PLANT J. vol. 10, no. 2, pp. 225-234 (1996)
ISSN: 0960-7412
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Agricultural and Environmental Biotechnology Abstracts

Malate synthase is a glyoxysome-specific enzyme. The carboxy-terminal tripeptide of the enzyme is Ser-Arg-Leu (SRL), which is known to function as a **peroxisomal targeting** signal in mammalian cells. To analyze the function of the carboxy-terminal amino acids of pumpkin malate synthase in **plant** cells, a chimeric gene was constructed that encoded a **fusion protein** which consisted of beta -glucuronidase and the carboxyl terminus of the enzyme. The **fusion protein** was **expressed** and accumulated in transgenic Arabidopsis that had been transformed with the chimeric gene. Immunocytochemical analysis of the transgenic **plants** revealed that the carboxy-terminal five amino acids of pumpkin malate synthase were sufficient for transport of the **fusion protein** into glyoxysomes in etiolated cotyledons, into leaf **peroxisomes** in green cotyledons and in mature leaves, and into unspecialized microbodies in roots, although the **fusion protein** was no longer transported into microbodies when SRL at the carboxyl terminus was deleted. Transport of **proteins** into glyoxysomes and leaf **peroxisomes** was also observed when the carboxy-terminal amino acids

of the **fusion protein** were changed from SRL to SKL, SRM, ARL or PRL. The results suggest that tripeptides with S, A or P at the -3 position, K or R at the -2 position, and L or M at the carboxyl terminal position can function as a **targeting** signal for three kinds of **plant** microbody. (DBO)

13/3,AB/32 (Item 2 from file: 76)
DIALOG(R)File 76:Life Sciences Collection
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02112990 4014702
Targeting and processing of a chimeric **protein** with the N-terminal presequence of the precursor to glyoxysomal citrate synthase
Kato, A.; Hayashi, M.; Kondo, M.; Nishimura, M.
Dep. Cell Biol., Natl. Inst. for Basic Biol., Okazaki 444, Japan
PLANT CELL vol. 8, no. 9, pp. 1601-1611 (1996)
ISSN: 1040-4651
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Biochemistry Abstracts 2: Nucleic Acids

Glyoxysomal citrate synthase in pumpkin is synthesized as a precursor that has a cleavable presequence at its N-terminal end. To investigate the role of the presequence in the transport of the **protein** to the microbodies, we generated transgenic Arabidopsis **plants** that **expressed** beta -glucuronidase with the N-terminal presequence of the precursor to the glyoxysomal citrate synthase of pumpkin. Immunogold labeling and cell fractionation studies showed that the chimeric **protein** was transported into microbodies and subsequently was processed. The chimeric **protein** was transported to functionally different microbodies, such as glyoxysomes, leaf **peroxisomes**, and unspecialized microbodies. These observations indicated that the transport of glyoxysomal citrate synthase is mediated by its N-terminal presequence and that the transport system is functional in all **plant** microbodies. Site-directed mutagenesis of the conserved amino acids in the presequence caused abnormal **targeting** and inhibition of processing of the chimeric **protein**, suggesting that the conserved amino acids in the presequence are required for recognition of the **target** or processing.

13/3,AB/33 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text
(c) 2001 The HW Wilson Co. All rts. reserv.

04504734 H.W. WILSON RECORD NUMBER: BGSA01004734
The genetics of **peroxisome** biogenesis.
Sacksteder, Katherine A
Gould, Stephen J
Annual Review of Genetics v. 34 (2000) p. 623-52
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 14079

ABSTRACT: The segregation of metabolic functions within discrete organelles is a hallmark of eukaryotic cells. These compartments allow for the concentration of related metabolic functions, the separation of competing metabolic functions, and the formation of unique chemical microenvironments. However, such organization is not spontaneous and requires an array of genes that are dedicated to the assembly and maintenance of these structures. In this review we focus on the genetics of **peroxisome** biogenesis and on how defects in this process cause human disease. Reprinted by permission of the publisher. Reprinted by permission of the publisher.

13/3,AB/34 (Item 2 [redacted]m file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04273996 H.W. WILSON RECORD NUMBER: BGSA00023996
Roles of the glutathione- and thioredoxin-dependent reduction systems in the Escherichia coli and Saccharomyces cerevisiae response to oxidative stress.
Carmel-Harel, Orna
Storz, Gisela
Annual Review of Microbiology v. 54 (2000) p. 439-61
SPECIAL FEATURES: bibl diag tab ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 9977

ABSTRACT: The glutathione- and thioredoxin-dependent reduction systems are responsible for maintaining the reduced environment of the Escherichia coli and Saccharomyces cerevisiae cytosol. Here we examine the roles of these two cellular reduction systems in the bacterial and yeast defenses against oxidative stress. The transcription of a subset of the genes encoding glutathione biosynthetic enzymes, glutathione reductases, glutaredoxins, thioredoxins, and thioredoxin reductases, as well as glutathione- and thioredoxin-dependent peroxidases is clearly induced by oxidative stress in both organisms. However, only some strains carrying mutations in single genes are hypersensitive to oxidants. This is due, in part, to the redundant effects of the gene products and the overlap between the two reduction systems. The construction of strains carrying mutations in multiple genes is helping to elucidate the different roles of glutathione and thioredoxin, and studies with such strains have recently revealed that these two reduction systems modulate the activities of the E. coli OxyR and SoxR and the S. cerevisiae Yap1p transcriptional regulators of the adaptive responses to oxidative stress. Reprinted by permission of the publisher.

13/3,AB/35 (Item 3 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04255636 H.W. WILSON RECORD NUMBER: BGSA00005636
Mitochondrial genome evolution and the origin of eukaryotes.
AUGMENTED TITLE: review
Lang, B. Franz
Gray, Michael W; Burger, Gertraud
Annual Review of Genetics v. 33 (1999) p. 351-97
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 19798

ABSTRACT: Recent results from ancestral (minimally derived) protists testify to the tremendous diversity of the mitochondrial genome in various eukaryotic lineages, but also reinforce the view that mitochondria, descendants of an endosymbiotic a-Proteobacterium, arose only once in evolution. The serial endosymbiosis theory, currently the most popular hypothesis to explain the origin of mitochondria, postulates the capture of an a-proteobacterial endosymbiont by a nucleus-containing eukaryotic host resembling extant amitochondriate protists. New sequence data have challenged this scenario, instead raising the possibility that the origin of the mitochondrion was coincident with, and contributed substantially to, the origin of the nuclear genome of the eukaryotic cell. Defining more precisely the a-proteobacterial ancestry of the mitochondrial genome, and the contribution of the endosymbiotic event to the nuclear genome, will be essential for a full understanding of the origin and evolution of the

eukaryotic cell as a whole. Reprinted by permission of the publisher.

13/3,AB/36 (Item 4 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04004129 H.W. WILSON RECORD NUMBER: BGSA99004129
Epitope tagging.
Jarvik, Jonathan W
Telmer, Cheryl A
Annual Review of Genetics v. 32 (1998) p. 601-18
SPECIAL FEATURES: bibl il ISSN: 0066-4197
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 8181

ABSTRACT: The use of epitope tagging, whereby a **protein** encoded by a cloned gene is rendered immunoreactive to a known antibody, is discussed. The method can be applied to the monitoring of **protein expression**, the localization of **proteins** at cellular and subcellular levels, **protein** purification, and the analysis of **protein** topology, dynamics, and interactions. The method can also be applied to transgenic and gene therapy studies and to the emerging fields of functional genomics and proteomics. Advantages and limitations of epitope tagging are summarized

13/3,AB/37 (Item 5 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03805285 H.W. WILSON RECORD NUMBER: BGSI98055285
Regulation of acetate metabolism by **protein** phosphorylation in enteric bacteria.
Cozzone, Alain J
Annual Review of Microbiology (Annu Rev Microbiol) v. 52 ('98) p. 127-64
SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 17608

ABSTRACT: Growth of enteric bacteria on acetate as the sole source of carbon and energy requires operation of a particular anaplerotic pathway known as the glyoxylate bypass. In this pathway, two specific enzymes, isocitrate lyase and malate synthase, are activated to divert isocitrate from the tricarboxylic acid cycle and prevent the quantitative loss of acetate carbons as carbon dioxide. Bacteria are thus supplied with the metabolic intermediates they need for synthesizing their cellular components. The channeling of isocitrate through the glyoxylate bypass is regulated via the phosphorylation/dephosphorylation of isocitrate dehydrogenase, the enzyme of the tricarboxylic acid cycle which competes for a common substrate with isocitrate lyase. When bacteria are grown on acetate, isocitrate dehydrogenase is phosphorylated and, concomitantly, its activity declines drastically. Conversely, when cells are cultured on a preferred carbon source, such as glucose, the enzyme is dephosphorylated and recovers full activity. Such reversible phosphorylation is mediated by an unusual bifunctional enzyme, isocitrate dehydrogenase kinase/phosphatase, which contains both modifying and demodifying activities on the same polypeptide. The genes coding for malate synthase, isocitrate lyase, and isocitrate dehydrogenase kinase/phosphatase are located in the same operon. Their **expression** is controlled by a complex dual mechanism that involves several transcriptional repressors and activators. Recent developments have brought new insights into the nature and mode of action of these different regulators. Also, significant

advances have been made lately in our understanding of the control of enzyme activity by reversible phosphorylation. In general, analyzing the physiological behavior of bacteria on acetate provides a valuable approach for deciphering at the molecular level the mechanisms of cell adaptation to the environment. With permission, from the Annual Review of Microbiology, Volume 52, 1998, by Annual Reviews Inc. (http://www.annurev.org).

13/3,AB/38 (Item 6 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03796064 H.W. WILSON RECORD NUMBER: BGSA98046064
The AMP-activated/SNF1 **protein** kinase subfamily: metabolic sensors of the eukaryotic cell?.
AUGMENTED TITLE: review
Hardie, D. Grahame
Carling, David; Carlson, Marian
Annual Review of Biochemistry v. 67 (1998) p. 821-55
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 15091

ABSTRACT: Mammalian AMP-activated **protein** kinase and yeast SNF1 **protein** kinase are the central components of kinase cascades that are highly conserved between animals, fungi, and **plants**. The AMP-activated **protein** kinase cascade acts as a metabolic sensor or "fuel gauge" that monitors cellular AMP and ATP levels because it is activated by increases in the AMP:ATP ratio. Once activated, the enzyme switches off ATP-consuming anabolic pathways and switches on ATP-producing catabolic pathways, such as fatty acid oxidation. The SNF1 complex in yeast is activated in response to the stress of glucose deprivation. In this case the intracellular signal or signals have not been identified; however, SNF1 activation is associated with depletion of ATP and elevation of AMP. The SNF1 complex acts primarily by inducing **expression** of genes required for catabolic pathways that generate glucose, probably by triggering phosphorylation of transcription factors. SNF1-related **protein** kinases in higher **plants** are likely to be involved in the response of **plant** cells to environmental and/or nutritional stress. With permission, from the Annual Review of Biochemistry Volume 67, 1998, by Annual Reviews Inc. (http://www.annurev.org).

13/3,AB/39 (Item 7 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03796055 H.W. WILSON RECORD NUMBER: BGSI98046055
The green fluorescent **protein**.
AUGMENTED TITLE: review
Tsien, Roger Y
Annual Review of Biochemistry (Annu Rev Biochem) v. 67 ('98) p. 509-44
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 13232

ABSTRACT: In just three years, the green fluorescent **protein** (GFP) from the jellyfish Aequorea victoria has vaulted from obscurity to become one of the most widely studied and exploited **proteins** in biochemistry and cell biology. Its amazing ability to generate a highly visible, efficiently emitting internal fluorophore is both intrinsically fascinating and tremendously valuable. High-resolution crystal structures of GFP offer unprecedented opportunities to understand and manipulate the relation

between **protein** structure and spectroscopic function. GFP has become well established as a marker of gene **expression** and **protein targeting** in intact cells and organisms. Mutagenesis and engineering of GFP into chimeric **proteins** are opening new vistas in physiological indicators, biosensors, and photochemical memories. With permission, from the Annual Review of Biochemistry Volume 67, 1998, by Annual Reviews Inc. (http://www.annurev.org).

13/3,AB/40 (Item 8 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

03546777 H.W. WILSON RECORD NUMBER: BGSI97046777
Protein import into mitochondria.
Neupert, Walter
Annual Review of Biochemistry (Annu Rev Biochem) v. 66 ('97) p. 863-917
SPECIAL FEATURES: bibl il ISSN: 0066-4154
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 26064

ABSTRACT: Mitochondria import many hundreds of different **proteins** that are encoded by nuclear genes. These **proteins** are **targeted** to the mitochondria, translocated through the mitochondrial membranes, and sorted to the different mitochondrial subcompartments. Separate translocases in the mitochondrial outer membrane (TOM complex) and in the inner membrane (TIM complex) facilitate recognition of preproteins and transport across the two membranes. Factors in the cytosol assist in **targeting** of preproteins. **Protein** components in the matrix partake in energetically driving translocation in a reaction that depends on the membrane potential and matrix-ATP. Molecular chaperones in the matrix exert multiple functions in translocation, sorting, folding, and assembly of newly imported **proteins**. With permission, from the Annual Review of Biochemistry Volume 66, 1997, by Annual Reviews Inc.

1/98

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? s polyhydroxyalkanoate?

      S1    1868  POLYHYDROXYALKANOATE?
? s s1 and peroxisome?

            1868  S1
           33171  PEROXISOME?
      S2      29  S1 AND PEROXISOME?
? rd

>>>Duplicate detection is not supported for File 306.

>>>Records from unsupported files will be retained in the RD set.
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? t s3/3,ab/all

>>>No matching display code(s) found in file(s): 65, 306

 3/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11195348   20532791   PMID: 11080293
  Analysis of the alternative pathways for the beta-oxidation of
unsaturated fatty acids using transgenic plants synthesizing
polyhydroxyalkanoates in peroxisomes.
  Allenbach L; Poirier Y
  Institut d'Ecologie-Biologie et Physiologie Vegetales, Batiment de
Biologie, Universite de Lausanne, CH-1015 Lausanne, Switzerland.
  Plant physiology (UNITED STATES) Nov 2000, 124 (3) p1159-68, ISSN
0032-0889 Journal Code: P98
  Languages: ENGLISH
  Document type: Journal Article
  Record type: Completed
  Degradation of fatty acids having cis-double bonds on even-numbered
carbons requires the presence of auxiliary enzymes in addition to the
enzymes of the core beta-oxidation cycle. Two alternative pathways have
been described to degrade these fatty acids. One pathway involves the
participation of the enzymes 2, 4-dienoyl-coenzyme A (CoA) reductase and
Delta(3)-Delta(2)-enoyl-CoA isomerase, whereas the second involves the
epimerization of R-3-hydroxyacyl-CoA via a 3-hydroxyacyl-CoA epimerase or
the action of two stereo-specific enoyl-CoA hydratases. Although
degradation of these fatty acids in bacteria and mammalian
peroxisomes was shown to involve mainly the reductase-isomerase
pathway, previous analysis of the relative activity of the enoyl-CoA
hydratase II (also called R-3-hydroxyacyl-CoA hydro-lyase) and
2,4-dienoyl-CoA reductase in plants indicated that degradation occurred
mainly through the epimerase pathway. We have examined the implication of
both pathways in transgenic Arabidopsis expressing the
polyhydroxyalkanoate synthase from Pseudomonas aeruginosa in
peroxisomes and producing polyhydroxyalkanoate from the
3-hydroxyacyl-CoA intermediates of the beta-oxidation cycle. Analysis of
the polyhydroxyalkanoate synthesized in plants grown in media
containing cis-10-heptadecenoic or cis-10-pentadecenoic acids revealed a
significant contribution of both the reductase-isomerase and epimerase
pathways to the degradation of these fatty acids.
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...completed examining records
S13 40 RD (unique items)
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1/98

>>>No matching display code(s) found in file(s): 65, 306

13/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11378204 21228804 PMID: 11330041
A leaf-**peroxisomal protein** , hydroxypyruvate reductase, is produced by light-regulated alternative splicing.
Mano S; Hayashi M; Nishimura M
Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.
Cell biochemistry and biophysics (United States) 2000, 32 Spring p147-54, ISSN 1085-9195 Journal Code: CKG
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Hydroxypyruvate reductase (HPR) is localized in leaf **peroxisomes** in **plants** , and it plays an important role in the glycolate pathway of photorespiration. In this laboratory, two highly homologous cDNAs for pumpkin HPR (HPR1 and HPR2) have been obtained, and appear to be produced from the same primary transcript by alternative splicing. Analyses at the mRNA level showed that the amounts of the two HPR mRNAs is changed in response to light, suggesting that light changes the splicing pattern of HPR pre-mRNA from almost equal amounts of two HPR mRNAs to greater production of HPR2 mRNA. From the sequences of the two HPR cDNAs, the HPR1 **protein**, but not the HPR2 **protein**, was found to have a **targeting** sequence into **peroxisomes** at the carboxy terminus. Analyses of transgenic Arabidopsis thaliana **expressing fusion proteins** with green fluorescent **protein** confirmed the different subcellular localizations of the two HPR **proteins**. These findings indicate the presence of light-regulated alternative splicing of HPR pre-mRNA, which controls the subcellular localizations of two HPR **proteins** in pumpkin cells.

13/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11242016 21151123 PMID: 11256623
Caenorhabditis elegans has a single pathway to **target** matrix **proteins** to **peroxisomes**.
Motley AM; Hettema EH; Ketting R; Plasterk R; Tabak HF
Department of Biochemistry, Academic Medical Center, Amsterdam, The Netherlands.
EMBO Rep (England) Jul 2000, 1 (1) p40-6, ISSN 1469-221X Journal Code: D0T
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
All eukaryotes so far studied, including animals, **plants**, yeasts and trypanosomes, have two pathways to **target proteins** to **peroxisomes**. These two pathways are specific for the two types of **peroxisome targeting** signal (PTS) present on **peroxisomal** matrix **proteins**. Remarkably, the complete genome sequence of Caenorhabditis elegans lacks the genes encoding **proteins** specific for the PTS2 **targeting** pathway. Here we show, by **expression** of green fluorescent **protein** (GFP) reporters for both pathways, that the PTS2 pathway is indeed absent in C. elegans. Lack of this pathway in man

causes severe disease due to mislocalization of PTS2-containing **proteins** . This raises the question as to how C. elegans has accommodated the absence of the PTS2 pathway. We found by in silico analysis that C. elegans orthologues of PTS2-containing **proteins** have acquired a PTS1. We propose that switching of **targeting** signals has allowed the PTS2 pathway to be lost in the phylogenetic lineage leading to C. elegans.

13/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10560600 20202702 PMID: 10737809
Random GFP::cDNA **fusions** enable visualization of subcellular structures in cells of Arabidopsis at a high frequency.
Cutler SR; Ehrhardt DW; Griffitts JS; Somerville CR
Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford CA 94305, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 28 2000, 97 (7) p3718-23, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We describe a general approach for identifying components of subcellular structures in a multicellular organism by exploiting the ability to generate thousands of independent transformants in Arabidopsis thaliana. A library of Arabidopsis cDNAs was constructed so that the cDNAs were inserted at the 3' end of the green fluorescent **protein** (GFP) coding sequence. The library was introduced en masse into Arabidopsis by Agrobacterium-mediated transformation. Fluorescence imaging of 5,700 transgenic **plants** indicated that approximately 2% of lines **expressed** a **fusion protein** with a different subcellular distribution than that of soluble GFP. About half of the markers identified were **targeted** to **peroxisomes** or other subcellular destinations by non-native coding sequence (i.e., out-of-frame cDNAs). This observation suggests that some **targeting** signals are of sufficiently low information content that they can be generated frequently by chance. The potential of the approach for identifying markers with unique dynamic processes is demonstrated by the identification of a GFP **fusion protein** that displays a cell-cycle regulated change in subcellular distribution. Our results indicate that screening GFP-**fusion protein** libraries is a useful approach for identifying and visualizing components of subcellular structures and their associated dynamics in higher **plant** cells.

13/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09926199 99007315 PMID: 9789089
Identification and analysis of the **plant peroxisomal targeting** signal 1 receptor NtPEX5.
Kragler F; Lametschwandtner G; Christmann J; Hartig A; Harada JJ
Section of Plant Biology, Division of Biological Sciences, University of California, One Shields Avenue, Davis, CA 95616, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Oct 27 1998, 95 (22) p13336-41, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Protein translocation into **peroxisomes** takes place via recognition of a **peroxisomal targeting** signal present at either the extreme C termini (PTS1) or N termini (PTS2) of matrix **proteins**.

In mammals and yeast, the **peroxisomal targeting** signal receptor, Pex5p, recognizes the PTS1 consisting of SKL or variants thereof. Although many **plant peroxisomal** matrix **proteins** are transported through the PTS1 pathway, little is known about the PTS1 receptor or any other **peroxisome** assembly **protein** from **plants** . We cloned tobacco (Nicotiana tabacum) cDNAs encoding Pex5p (NtPEX5) based on the **protein**'s interaction with a PTS1-containing **protein** in the yeast two-hybrid system. Nucleotide sequence analysis revealed that the tobacco Pex5p contains seven tetratricopeptide repeats and that NtPEX5 shares greater sequence similarity with its homolog from humans than from yeast. **Expression** of NtPEX5 **fusion proteins** , consisting of the N-terminal part of yeast Pex5p and the C-terminal region of NtPEX5, in a Saccharomyces cerevisiae pex5 mutant restored **protein** translocation into **peroxisomes** . These experiments confirmed the identity of the tobacco **protein** as a PTS1 receptor and indicated that components of the **peroxisomal** translocation apparatus are conserved functionally. Two-hybrid assays showed that NtPEX5 interacts with a wide range of PTS1 variants that also interact with the human Pex5p. Interestingly, the C-terminal residues of some of these peptides deviated from the established **plant** PTS1 consensus sequence. We conclude that there are significant sequence and functional similarities between the **plant** and human Pex5ps.

13/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09517446 96367666 PMID: 8771780
Transport of chimeric **proteins** that contain a carboxy-terminal **targeting** signal into **plant** microbodies.
Hayashi M; Aoki M; Kato A; Kondo M; Nishimura M
Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan.
Plant journal (ENGLAND) Aug 1996, 10 (2) p225-34, ISSN 0960-7412 Journal Code: BRU
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Malate synthase is a glyoxysome-specific enzyme. The carboxy-terminal tripeptide of the enzyme is Ser-Arg-Leu (SRL), which is known to function as a **peroxisomal targeting** signal in mammalian cells. To analyze the function of the carboxy-terminal amino acids of pumpkin malate synthase in **plant** cells, a chimeric gene was constructed that encoded a **fusion protein** which consisted of beta-glucuronidase and the carboxyl terminus of the enzyme. The **fusion protein** was **expressed** and accumulated in transgenic Arabidopsis that had been transformed with the chimeric gene. Immunocytochemical analysis of the transgenic **plants** revealed that the carboxy-terminal five amino acids of pumpkin malate synthase were sufficient for transport of the **fusion protein** into glyoxysomes in etiolated cotyledons, into leaf **peroxisomes** in green cotyledons and in mature leaves, and into unspecialized microbodies in roots, although the **fusion protein** was no longer transported into microbodies when SRL at the carboxyl terminus was deleted. Transport of **proteins** into glyoxysomes and leaf **peroxisomes** was also observed when the carboxy-terminal amino acids of the **fusion protein** were changed from SRL to SKL, SRM, ARL or PRL. The results suggest that tripeptides with S, A or P at the -3 position, K or R at the -2 position, and L or M at the carboxyl terminal position can function as a **targeting** signal for three kinds of **plant** microbody.

13/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09517406 96400038 PM[redacted] 8806414
cDNA cloning and **exp[redacted]ssion** of a gene for 3-ketoacyl[redacted]A thiolase in pumpkin cotyledons.
Kato A; Hayashi M; Takeuchi Y; Nishimura M
Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan.
Plant molecular biology (NETHERLANDS) Jul 1996, 31 (4) p843-52, ISSN 0167-4412 Journal Code: A6O
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
A cDNA clone for 3-ketoacyl-CoA thiolase (EC 2.3.1.16) was isolated from a lambda gt11 cDNA library constructed from the poly(A)+ RNA of etiolated pumpkin cotyledons. The cDNA insert contained 1682 nucleotides and encoded 461 amino acid residues. A study of the **expression** in vitro of the cDNA and analysis of the amino-terminal sequence of the **protein** indicated that pumpkin thiolase is synthesized as a precursor which has a cleavable amino-terminal presequence of 33 amino acids. The amino-terminal presequence was highly homologous to typical amino-terminal signals that **target proteins** to microbodies. Immunoblot analysis showed that the amount of thiolase increased markedly during germination but decreased dramatically during the light-inducible transition of microbodies from glyoxysomes to leaf **peroxisomes** . By contrast, the amount of mRNA increased temporarily during the early stage of germination. In senescing cotyledons, the levels of the thiolase mRNA and **protein** increased again with the reverse transition of microbodies from leaf **peroxisomes** to glyoxysomes, but the pattern of accumulation of the **protein** was slightly different from that of malate synthase. These results indicate that **expression** of the thiolase is regulated in a similar manner to that of other glyoxysomal enzymes, such as malate synthase and citrate synthase, during seed germination and post-germination growth. By contrast, during senescence, **expression** of the thiolase is regulated in a different manner from that of other glyoxysomal enzymes.

13/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08353826 95179791 PMID: 7874730
Stabilization of methionine-rich **protein** in Saccharomyces cerevisiae: **targeting** of BZN **protein** into the **peroxisome**.
Nicaud JM; Raynal A; Beyou A; Merkamm M; Ito H; Labat N
Laboratoire de recherche d'EUROLYSINE, Parc club Orsay Universite, France.
Current genetics (UNITED STATES) Nov-Dec 1994, 26 (5-6) p390-7, ISSN 0172-8083 Journal Code: CUG
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We have constructed a gene coding for the 12-kDa intermediate form of the 2s methionine-rich **protein** from Bertholletia excelsa seeds. This **protein, expressed** intracellularly in yeast, is characterised by a 20-min half-life. By adding 11 amino acids corresponding to the **peroxisome-targeting** sequence (PTSc) of luciferase, we have significantly increased its half-life. This stabilization allowed accumulation of the BZN **protein** into the **peroxisome** as judged by cell fractionation. Accumulation of the 12-kDa **protein** results in a significant increase of the total methionine content in yeast cells (30%) indicating that such a microorganism could represent a practicable protected shuttle for an animal-feed additive.

13/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08295530 95074153 PM[redacted]. 7983054
Molecular cloning and characterization of a cDNA encoding pea monodehydroascorbate reductase.
Murthy SS; Zilinskas BA
Department of Plant Science, Cook College, Rutgers University, New Brunswick, New Jersey 08903-0231.
Journal of biological chemistry (UNITED STATES) Dec 9 1994, 269 (49) p31129-33, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Monodehydroascorbate radicals are generated in **plant** cells enzymatically by the hydrogen peroxide scavenging enzyme, ascorbate peroxidase, and nonenzymatically via the univalent oxidation of ascorbate by superoxide, hydroxyl, and various organic radicals. Regeneration of ascorbate is achieved by monodehydroascorbate reductase (EC 1.6.5.4) using NAD(P)H as an electron donor or, alternatively, by a set of two coupled reactions requiring dehydroascorbate reductase, glutathione reductase, glutathione, and NAD(P)H. As monodehydroascorbate reductase is a key enzyme in maintaining reduced pools of ascorbate, an important antioxidant, we undertook this study to learn more about its structure, function, and regulation. Herein we report the molecular cloning and characterization of a cDNA encoding monodehydroascorbate reductase of pea (Pisum sativum L.). The cDNA encodes a 433-amino acid polypeptide that shows, respectively, 73 and 87% identity with peptide fragments from soybean and cucumber monodehydroascorbate reductase. Monodehydroascorbate reductase contains the NAD(P)H and FAD binding domains of other flavin oxidoreductases. The cloned enzyme lacks a transit peptide, but the sequence of the carboxyl terminus is Ser-Lys-Ile, similar to the **targeting** motif found in **peroxisomal proteins**. When **expressed** in Escherichia coli fused to maltose-binding **protein**, monodehydroascorbate reductase has enzymatic properties comparable with purified soybean and cucumber monodehydroascorbate reductase. Northern blot analysis shows that the monodehydroascorbate reductase transcript is 1.6 kilobase in size and is **expressed** at relatively low levels in all **plant** tissues examined.

13/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08039931 93320378 PMID: 8329679
Targeting of castor bean glyoxysomal isocitrate lyase to tobacco leaf **peroxisomes**.
Onyeocha I; Behari R; Hill D; Baker A
Department of Biochemistry, University of Cambridge, UK.
Plant molecular biology (NETHERLANDS) Jun 1993, 22 (3) p385-96, ISSN 0167-4412 Journal Code: A6O
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The cDNA encoding castor bean endosperm isocitrate lyase (ICL) was **expressed** under the control of the promoter of the small subunit of pea ribulose bisphosphate carboxylase in transformed tobacco. ICL **protein** was detected using anti-ICL antibodies on immunoblots of total leaf **protein** extracts. Nycodenz density gradient separation of the extracts from the transgenic tobacco leaves showed ICL co-fractionated with hydroxypyruvate reductase, a **peroxisomal** matrix marker **protein**, and away from lactate dehydrogenase, a cytosolic marker **protein** . Immunoelectron microscopy of ultrathin leaf sections demonstrated the location of ICL within the matrix of the leaf **peroxisomes** of the transgenic **plants**. In vitro transcribed and translated ICL was also imported into leaf **peroxisomes** isolated from germinating sunflower seeds. The in vivo and in vitro import of this

protein into leaf **peroxisomes** provides strong support for the notion that the import machinery of glyoxysomes and **peroxisomes** is very similar.

13/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07544906 93251103 PMID: 1844888
The carboxy-terminal end of glycolate oxidase directs a foreign **protein** into tobacco leaf **peroxisomes**.
Volokita M
Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel.
Plant journal (ENGLAND) Nov 1991, 1 (3) p361-6, ISSN 0960-7412 Journal Code: BRU
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The carboxy-terminal residues of several **peroxisomal proteins** were shown to act as a **peroxisomal targetting** signal. This study was conducted to test whether the C-terminus of glycolate oxidase, a key enzyme in the glycolate metabolism pathway, is functioning as a **targetting** signal that directs **proteins** into **plant** leaf **peroxisomes**. A chimeric gene coding for a **fusion protein** composed of the C-terminal-truncated beta-glucuronidase, a synthetic linker of four amino acids and the last six C-terminal amino acids of glycolate oxidase, was constructed. Transformation of tobacco **plants** with the chimeric gene resulted in **expression** of beta-glucuronidase enzymic activity. About 50% of the transgenic beta-glucuronidase activity was localized to the **peroxisomes** . The results indicate that the six C-terminal amino acid residues of glycolate oxidase act as a **targetting** signal that is recognized by leaf **peroxisomes**.

13/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07171525 92109785 PMID: 1764107
Amino-terminal presequence of the precursor of **peroxisomal** 3-ketoacyl-CoA thiolase is a cleavable signal peptide for **peroxisomal targeting**.
Osumi T; Tsukamoto T; Hata S; Yokota S; Miura S; Fujiki Y; Hijikata M; Miyazawa S; Hashimoto T
Department of Life Science, Himeji Institute of Technology, Hyogo, Japan.
Biochemical and biophysical research communications (UNITED STATES) Dec 31 1991, 181 (3) p947-54, ISSN 0006-291X Journal Code: 9Y8
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
To examine the function of the amino-terminal presequence of rat **peroxisomal** 3-ketoacyl-CoA thiolase precursor, **fusion proteins** of various amino-terminal regions of the precursor with non-**peroxisomal** enzymes were **expressed** in cultured mammalian cells. On immunofluorescence microscopy, all constructs carrying the presequence part exhibited punctate patterns of distribution, identical with that of catalase, a **peroxisomal** marker. **Proteins** lacking all or a part of the prepiece were found in the cytosol. These results indicate that the presequence of the thiolase has sufficient information for **peroxisomal targeting**.

13/3,AB/12 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13059180 BIOSIS NO.: 200100266329
Peroxisomal remnant structures in Hansenula polymorpha pex5 cells can develop into normal **peroxisomes** upon induction of the PTS2 **protein** amine oxidase.
AUTHOR: Salomons Florian A; Faber Klaas Nico; Veenhuis Marten; van der Klei Ida J(a)
AUTHOR ADDRESS: (a)Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9750 AA, Haren: I.J.van.der.Klei@biol.rug.nl**Netherlands
JOURNAL: Journal of Biological Chemistry 276 (6):p4190-4198 February 9, 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: We have analyzed the properties of **peroxisomal** remnants in Hansenula polymorpha pex5 cells. In such cells PTS1 matrix **protein** import is fully impaired. In H. polymorpha pex5 cells, grown on ethanol/ammonium sulfate, conditions that repressed the PTS2 **protein** amine oxidase (AMO), **peroxisomal** structures were below the limit of detection. In methanol/ammonium sulfate-grown cells, normal **peroxisomes** are absent, but a few small membranous structures were observed that apparently represented **peroxisomal** ghosts since they contained Pex14p. These structures were the **target** of a Pex10p.myc **fusion protein** that was produced in pex5 cells under the control of the homologous alcohol oxidase promoter (strain pex5::PAOX.PEX10.MYC). Glycerol/methanol/ammonium sulfate-grown cells of this transformant were placed in fresh glucose/methylamine media, conditions that fully repress the synthesis of the Pex10p.myc **fusion protein** but induce the synthesis of AMO. Two hours after the shift Pex10p.myc-containing structures were detectable that had accumulated newly synthesized AMO **protein** and which during further cultivation developed in normal **peroxisomes**. Concurrently, the remaining portion of these structures was rapidly degraded. These findings indicate that **peroxisomal** remnants in pex5 cells can develop into **peroxisomes**. Also, as for normal **peroxisomes** in H. polymorpha, apparently a minor portion of these structures actually take part in the development of these organelles.

2001

13/3,AB/13 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13036756 BIOSIS NO.: 200100243905
Discrete **targeting** signals direct Pmp47 to oleate-induced **peroxisomes** in Saccharomyces cerevisiae.
AUTHOR: Wang Xiaodong; Unruh Michael J; Goodman Joel M(a)
AUTHOR ADDRESS: (a)Dept. of Pharmacology, University of Texas Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, TX, 75390-9041: Joel.Goodman@UTSouthwestern.edu**USA
JOURNAL: Journal of Biological Chemistry 276 (14):p10897-10905 April 6, 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Pmp47 is a **peroxisomal** membrane **protein** consisting of six transmembrane domains (TMDs). We previously showed that the second matrix loop containing a basic cluster of amino acids is important for **peroxisomal targeting**, and similar basic **targeting** motifs have been found in other **peroxisomal** membrane **proteins**. However, this basic cluster by itself **targets** to **peroxisomes** very poorly. We have developed a sensitive quantitative localization assay based on the **targeting** of Pmp47-GFP **fusion proteins** to identify the important elements of the basic cluster and to search for other **targeting** information on Pmp47. Our data suggest that side-chain structure and position as well as charge are important for **targeting** by the basic cluster. Analysis of other regions of Pmp47 indicates that all TMDs except TMD2 can be eliminated or substituted without significant loss of **targeting**. TMD2 plus an adjacent cytoplasmic-oriented sequence is crucial for **targeting**. Cytoplasmic-oriented sequences from two other **peroxisomal** membrane **proteins**, ScPex15p and ScPmp22, could partially substitute for the analogous sequence in Pmp47. **Targeting** with high fidelity to oleate-induced **peroxisomes** required the following elements: the cytoplasmic-oriented sequence and TMD2, a short matrix loop containing a basic cluster, and a membrane-anchoring TMD.

2001

13/3,AB/14 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12565674 BIOSIS NO.: 200000319176
A new self-assembled **peroxisomal** vesicle required for efficient resealing of the plasma membrane.
AUTHOR: Jedd Gregory; Chua Nam-Hai
AUTHOR ADDRESS: (a)Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY, 10021-6399**USA
JOURNAL: Nature Cell Biology 2 (4):p226-231 April, 2000
MEDIUM: print
ISSN: 1465-7392
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: The Woronin body is a membrane-bound organelle that has been observed in over 50 species of filamentous fungi. However, neither the composition nor the precise function of the Woronin body has yet been determined. Here we purify the Woronin body from Neurospora crassa and isolate Hex1, a new **protein** containing a consensus sequence known as **peroxisome-targeting** signal-1 (PTS1). We show that Hex1 is localized to the matrix of the Woronin body by immunoelectron microscopy, and that a green fluorescent **protein**- (GFP-)Hex1 **fusion protein** is **targeted** to yeast **peroxisomes** in a PTS1- and peroxin-dependent manner. The **expression** of the HEX1 gene in yeast generates hexagonal vesicles that are morphologically similar to the native Woronin body, implying a Hex1-encoded mechanism of Woronin-body assembly. Deletion of HEX1 in N. crassa eliminates Woronin bodies from the cytoplasm and results in hyphae that exhibit a cytoplasmic-bleeding phenotype in response to cell lysis. Our results show that the Woronin body represents a new category of **peroxisome** with a function in the maintenance of cellular integrity.

2000

13/3,AB/15 (Item 4 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)
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11920529 BIOSIS NO.: 199900166638
Light regulates alternative splicing of hydroxypyruvate reductase in pumpkin.
AUTHOR: Mano Shoji; Hayashi Makoto; Nishimura Mikio(a)
AUTHOR ADDRESS: (a)Dep. Cell Biol., National Inst. Basic Biol., Okazaki 444-8585**Japan
JOURNAL: Plant Journal 17 (3):p309-320 Feb., 1999
ISSN: 0960-7412
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Hydroxypyruvate reductase (HPR) is a leaf **peroxisomal** enzyme that functions in the glycolate pathway of photorespiration in **plants**. We have obtained two highly similar cDNAs for pumpkin HPR (HPR1 and HPR2). It has been revealed that two HPR mRNAs might be produced by alternative splicing from a single type of pre-mRNA. The HPR1 **protein**, but not the HPR2 **protein**, was found to have a **targeting** sequence into leaf **peroxisomes** at the C-terminus, suggesting that alternative splicing controls the subcellular localization of the two HPR **proteins**. Immunoblot analysis and subcellular fractionation experiments showed that HPR1 and HPR2 **proteins** are localized in leaf **peroxisomes** and the cytosol, respectively. Moreover, indirect fluorescence microscopy and analyses of transgenic tobacco cultured cells and Arabidopsis thaliana **expressing fusion proteins** with green fluorescent **protein** (GFP) revealed the different subcellular localizations of the two HPR **proteins**. Both mRNAs were induced developmentally and by light, but with quantitative differences. Almost equal amounts of the mRNAs were detected in pumpkin cotyledons grown in darkness, but treatment with light greatly enhanced the production of HPR2 mRNA. These findings indicate that light regulates alternative splicing of HPR mRNA, suggesting the presence of a novel mechanism of mRNA maturation, namely light-regulated alternative splicing, in higher **plants**.

1999

13/3,AB/16 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

11179355 BIOSIS NO.: 199799800500
Isocitrate lyase localisation in Saccharomyces cerevisiae cells.
AUTHOR: Chaves Romina S; Herrero Pilar; Ordiz Isabel; Del Brio Maria Angeles; Moreno Fernando(a)
AUTHOR ADDRESS: (a)Departamento de Bioquimica y Biologia Molecular, Instituto Universitario de Biotecnologia de Ast**Spain
JOURNAL: Gene (Amsterdam) 198 (1-2):p165-169 1997
ISSN: 0378-1119
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The isocitrate lyase from Saccharomyces cerevisiae was only located in the cell cytoplasm. This **protein** was found not to be associated with cell organelles, even under growth conditions that induce **peroxisome** proliferation. This conclusion is supported by experiments carried out by damaging the protoplast plasma membrane with DEAE-dextran, by differential centrifugation of osmotically lysed protoplast and by using the green fluorescent **protein** (GFP) of Aequorea victoria as a reporter **fusion** tag to localize the subcellular compartment to which isocitrate lyase is **targeted**.

13/3,AB/17 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10561948 BIOSIS NO.: 199699183093
Targeting of human catalase to **peroxisomes** is dependent upon a novel COOH-terminal **peroxisomal targeting** sequence.
AUTHOR: Purdue P Edward(a); Lazarow Paul B
AUTHOR ADDRESS: (a)Dep. Cell Biol. Anat., Box 1007, Mt. Sinai Sch. Med., One Gustave Levy Place, New York, NY 10029**USA
JOURNAL: Journal of Cell Biology 134 (4):p849-862 1996
ISSN: 0021-9525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have identified a novel **peroxisomal targeting** sequence (PTS) at the extreme COOH terminus of human catalase. The last four amino acids of this **protein** (-KANL) are necessary and sufficient to effect **targeting** to **peroxisomes** in both human fibroblasts and Saccharomyces cerevisiae. when appended to the COOH terminus of the reporter **protein**, chloramphenicol acetyl transferase. However, this PTS differs from the extensive family of COOH-terminal PTS tripeptides collectively termed PTS1 in two major aspects. First, the presence of the uncharged amino acid, asparagine, at the penultimate residue of the human catalase PTS is highly unusual, in that a basic residue at this position has been previously found to be a common and critical feature of PTS1 signals. Nonetheless, this asparagine residue appears to constitute an important component of the catalase PTS. in that replacement with aspartate abolished **peroxisomal targeting** (as did deletion of the COOH-terminal four residues). Second, the human catalase PTS comprises more than the COOH-terminal three amino acids, in that COOH-terminal-ANL cannot functionally replace the PTS1 signal-SKL in **targeting** a chloramphenicol acetyl transferase **fusion protein to peroxisomes**. The critical nature of the fourth residue from the COOH terminus of the catalase PTS (lysine) is emphasized by the fact that substitution of this residue with a variety of other amino acids abolished or reduced **peroxisomal targeting**. **Targeting** was not reduced when this lysine was replaced with arginine. suggesting that a basic amino acid at this position is required for maximal functional activity of this PTS. In spite of these unusual features, human catalase is sorted by the PTS1 pathway, both in yeast and human cells. Disruption of the PAS10 gene encoding the S. cerevisiae PTS1 receptor resulted in a cytosolic location of chloramphenicol acetyl transferase appended with the human catalase PTS, as did **expression** of this **protein** in cells from a neonatal adrenoleukodystrophy patient specifically defective in PTS1 import. Furthermore, through the use of the two-hybrid system, it was demonstrated that both the PAS10 gene product (Pas10p) and the human PTS1 receptor can interact with the COOH-terminal region of human catalase, but that this interaction is abolished by substitutions at the penultimate residue (asparagine-to-aspartate) and at the fourth residue from the COOH terminus (lysine-to-glycine) which abolish PTS functionality. We have found no evidence of additional **targeting** information elsewhere in the human catalase **protein**. An internal tripeptide (-SHL-, which conforms to the mammalian PTS1 consensus) located nine to eleven residues from the COOH terminus has been excluded as a functional PTS. Additionally, in contrast to the situation for S. cerevisiae catalase A. which contains an internal PTS in addition to a COOH-terminal PTS1, human catalase lacks such a redundant PTS, as evidenced by the exclusive cytosolic location of human catalase mutated in the COOH-terminal PTS. Consistent with this species difference,

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10771678 20040049 PMID: 10571864
Polyhydroxyalkanoate synthesis in transgenic plants as a new tool to study carbon flow through beta-oxidation.
Mittendorf V; Bongcam V; Allenbach L; Coullerez G; Martini N; Poirier Y
Institut dEcologie-Biologie et Physiologie Vegetales, Universite de Lausanne, CH-1015, Lausanne, Switzerland.
Plant journal (ENGLAND) Oct 1999, 20 (1) p45-55, ISSN 0960-7412 Journal Code: BRU
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Transgenic plants producing peroxisomal polyhydroxy- alkanoate (PHA) from intermediates of fatty acid degradation were used to study carbon flow through the beta-oxidation cycle. Growth of transgenic plants in media containing fatty acids conjugated to Tween detergents resulted in an increased accumulation of PHA and incorporation into the polyester of monomers derived from the beta-oxidation of these fatty acids. Tween-laurate was a stronger inducer of beta-oxidation, as measured by acyl-CoA oxidase activity, and a more potent modulator of PHA quantity and monomer composition than Tween-oleate. Plants co-expressing a peroxisomal PHA synthase with a capryl-acyl carrier protein thioesterase from Cuphea lanceolata produced eightfold more PHA compared to plants expressing only the PHA synthase. PHA produced in double transgenic plants contained mainly saturated monomers ranging from 6 to 10 carbons, indicating an enhanced flow of capric acid towards beta-oxidation. Together, these results support the hypothesis that plant cells have mechanisms which sense levels of free or esterified unusual fatty acids, resulting in changes in the activity of the beta-oxidation cycle as well as removal and degradation of these unusual fatty acids through beta-oxidation. Such enhanced flow of fatty acids through beta-oxidation can be utilized to modulate the amount and composition of PHA produced in transgenic plants. Furthermore, synthesis of PHAs in plants can be used as a new tool to study the quality and relative quantity of the carbon flow through beta-oxidation as well as to analyse the degradation pathway of unusual fatty acids.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10460729 20063345 PMID: 10594123
Increased flow of fatty acids toward beta-oxidation in developing seeds of Arabidopsis deficient in diacylglycerol acyltransferase activity or synthesizing medium-chain-length fatty acids.
Poirier Y; Ventre G; Caldelari D
Institut d'Ecologie-Biologie et Physiologie Vegetales, Batiment de Biologie, Universite de Lausanne, CH-1015 Lausanne, Switzerland. yves.poirier@ie-bpv.unil.ch
Plant physiology (UNITED STATES) Dec 1999, 121 (4) p1359-66, ISSN 0032-0889 Journal Code: P98
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Synthesis of **polyhydroxyalkanoates** (PHAs) from intermediates of fatty acid beta-oxidation was used as a tool to study fatty acid degradation in developing seeds of Arabidopsis. Transgenic plants expressing a peroxisomal PHA synthase under the control of a napin promoter accumulated PHA in developing seeds to a final level of 0. 06 mg g(-1) dry weight. In plants co-expressing a plastidial acyl-acyl carrier protein thioesterase from Cuphea lanceolata and a peroxisomal PHA synthase, approximately 18-fold more PHA accumulated in developing seeds. The proportion of 3-hydroxydecanoic acid monomer in the PHA was strongly increased, indicating a large flow of capric acid toward beta-oxidation.

Furthermore, expression of the peroxisomal PHA synthase in an Arabidopsis mutant deficient in the enzyme diacylglycerol acyltransferase resulted in a 10-fold increase in PHA accumulation in developing seeds. These data indicate that plants can respond to the inadequate incorporation of fatty acids into triacylglycerides by recycling the fatty acids via beta-oxidation and that a considerable flow toward beta-oxidation can occur even in a plant tissue primarily devoted to the accumulation of storage lipids.

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10431083 20054066 PMID: 10585189
Peroxisomes as sites for synthesis of **polyhydroxyalkanoates** in transgenic plants.
Hahn JJ; Eschenlauer AC; Sleytr UB; Somers DA; Srienc F
Department of Chemical Engineering, Biological Process Technology Institute, University of Minnesota, St. Paul, Minnesota 55108, USA.
Biotechnology progress (UNITED STATES) Nov-Dec 1999, 15 (6) p1053-7, ISSN 8756-7938 Journal Code: ALG
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Bacterial genes responsible for poly(3-hydroxybutyrate) (PHB) biosynthesis were targeted to plant **peroxisomes** by adding a carboxy-terminal targeting sequence. The enzymes evidently were transported into **peroxisomes**, retained their catalytic activity, and reacted with peroxisomally available precursors because PHB synthesis in transgenic plant cells was localized to **peroxisomes**. Up to 2 mg/g fresh weight PHB was produced in suspension cultures of Black Mexican Sweet maize cells after biolistic transformation with three peroxisomally targeted bacterial genes. An equilibrium effect is proposed to explain the unexpected existence of (R)-3-hydroxybutyryl-CoA in plant **peroxisomes**.

3/3,AB/5 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11754564 BIOSIS NO.: 199900000673
Synthesis of medium-chain-length polyhdyroxyalkanoates in Arabidopsis thaliana using intermediates of peroxisomal fatty acid beta-oxidation.
AUTHOR: Mittendorf Volker; Robertson Elizabeth J; Leech Rachel M; Krueger Niels; Steinbuechel Alexander; Poirier Yves(a)
AUTHOR ADDRESS: (a)Inst. Biol. Physiol. Vegetales, Batiment Biol., Univ. Lausanne, CH-1015 Lausanne**Switzerland
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 95 (23):p13397-13402 Nov. 10, 1998
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: **Polyhydroxyalkanoate** (PHA) is a family of polymers composed primarily of R-3-hydroxyalkanoic acids. These polymers have properties of biodegradable thermoplastics and elastomers. Medium-chain-length PHAs (MCL-PHAs) are synthesized in bacteria by using intermediates of the beta-oxidation of alkanoic acids. To assess the feasibility of producing MCL-PHAs in plants, Arabidopsis thaliana was transformed with the PhaC1 synthase from Pseudomonas aeruginosa modified for **peroxisome** targeting by addition of the carboxyl 34 amino acids from the Brassica napus isocitrate lyase. Immunocytochemistry demonstrated that the modified PHA synthase was appropriately targeted to leaf-type **peroxisomes** in light-grown plants and glyoxysomes in dark-grown

plants. Plants expressing the PHA synthase accumulated electron-lucent inclusions in the glyoxysomes and leaf-type **peroxisomes** as well as in the vacuole. These inclusions were similar to bacterial PHA inclusions. Analysis of plant extracts by GC and mass spectrometry demonstrated the presence of MCL-PHA in transgenic plants to approximately 4 mg per g of dry weight. The plant PHA contained saturated and unsaturated 3-hydroxyalkanoic acids ranging from six to 16 carbons with 41% of the monomers being 3-hydroxyoctanoic acid and 3-hydroxyoctenoic acid. These results indicate that the beta-oxidation of plant fatty acids can generate a broad range of R-3-hydroxyacyl-CoA intermediates that can be used to synthesize MCL-PHAs.

1998

3/3,AB/6 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3748931 21982089 Holding Library: AGL
Synthesis of medium-chain-length **polyhydroxyalkanoates** in Arabidopsis thaliana using intermediates of peroxisomal fatty acid beta-oxidation
Mittendorf, V. Robertson, E.J.; Leech, R.M.; Kruger, N.; Steinbuchel, A. ; Poirier, Y.
Universite de Lausanne, Lausanne, Switzerland.
Washington, D.C. : National Academy of Sciences,
Proceedings of the National Academy of Sciences of the United States of America. Nov 10, 1998. v. 95 (23) p. 13397-13402.
ISSN: 0027-8424 CODEN: PNASA6
DNAL CALL NO: 500 N21P
Language: English
Polyhydroxyalkanoate (PHA) is a family of polymers composed primarily of R-3-hydroxyalkanoic acids. These polymers have properties of biodegradable thermoplastics and elastomers. Medium-chain-length PHAs (MCL-PHAs) are synthesized in bacteria by using intermediates of the beta-oxidation of alkanoic acids. To assess the feasibility of producing MCL-PHAs in plants, Arabidopsis thaliana was transformed with the PhaC1 synthase from Pseudomonas aeruginosa modified for **peroxisome** targeting by addition of the carboxyl 34 amino acids from the Brassica napus isocitrate lyase. Immunocytochemistry demonstrated that the modified PHA synthase was appropriately targeted to leaf-type **peroxisomes** in light-grown plants and glyoxysomes in dark-grown plants. Plants expressing the PHA synthase accumulated electron-lucent inclusions in the glyoxysomes and leaf-type **peroxisomes** , as well as in the vacuole. These inclusions were similar to bacterial PHA inclusions. Analysis of plant extracts by GC and mass spectrometry demonstrated the presence of MCL-PHA in transgenic plants to approximately 4 mg per g of dry weight. The plant PHA contained saturated and unsaturated 3-hydroxyalkanoic acids ranging from six to 16 carbons with 41% of the monomers being 3-hydroxyoctanoic acid and 3-hydroxyoctenoic acid. These results indicate that the beta-oxidation of plant fatty acids can generate a broad range of R-3-hydroxyacyl-CoA intermediates that can be used to synthesize MCL-PHAs.

3/3,AB/7 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04114167 Genuine Article#: RE284 Number of References: 91
Title: STRATEGIES FOR THE SUSTAINABLE PRODUCTION OF NEW BIODEGRADABLE POLYESTERS IN PLANTS - A REVIEW (Abstract Available)
Author(s): VANDERLEIJ FR; WITHOLT B
Corporate Source: UNIV GRONINGEN HOSP,DEPT PEDIAT CARDIOL,POB 30001/9700 RB GRONINGEN//NETHERLANDS/; ETH ZURICH,HPT,INST BIOTECHNOL/CH-8093

ZURICH//SWITZERLAND/
Journal: CANADIAN JOURNAL OF MICROBIOLOGY, 1995, V41, S1, 22-238
ISSN: 0008-4166
Language: ENGLISH Document Type: ARTICLE
Abstract: In this study we review relevant pathways with regard to the production of poly(3-hydroxyalkanoates) (PHA) with medium chain length monomers in higher plants. On the basis of what is known of the genetics and the biochemistry of PHA formation in bacteria, and of fatty acid metabolism in various organisms, a number of possibilities for PHA production in model plants and in economically important crop plants are listed. Along with the molecular biology of PHA synthesis and fatty acid metabolism, we discuss theoretical and environmental considerations, metabolic engineering strategies, and plant transformation systems.

3/3,AB/8 (Item 1 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04053214 H.W. WILSON RECORD NUMBER: BGSA99053214
Prokaryotes.
Hoppert, Michael
Mayer, Frank
American Scientist (Am Sci) v. 87 no6 (Nov./Dec. 1999) p. 518-25
SPECIAL FEATURES: bibl il ISSN: 0003-0996
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 4946

ABSTRACT: The writer discusses prokaryotes, bacterial cells, and the differences between them and eukaryotes, the cells that make up plants and animals. Prokaryotes do not have membranous organelles and a normal eukaryotic cytoskeleton. Consequently, biomolecules are thought to be scattered randomly throughout the cytoplasm of these cells. However, prokaryotes display a high degree of subcellular organization. The operation and construction of these cells are detailed, and a new approach to looking at their actions is proposed.

3/3,AB/9 (Item 2 from file: 98)
DIALOG(R)File 98:General Sci Abs/Full-Text

04003057 H.W. WILSON RECORD NUMBER: BGSI99003057
Synthesis of medium-chain-length **polyhydroxyalkanoates** in Arabidopsis thaliana using intermediates of peroxisomal fatty acid b-oxidation.
Mittendorf, Volker
Robertson, Elizabeth J; Leech, Rachel M
Proceedings of the National Academy of Sciences of the United States of America (Proc Natl Acad Sci U S A) v. 95 no23 (Nov. 10 '98) p. 13397-402
SPECIAL FEATURES: bibl il ISSN: 0027-8424
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

ABSTRACT: The feasibility of producing medium-chain-length **polyhydroxyalkanoates** (PHAs) in plants was investigated. PHAs are a family of bacterial polymers that have the properties of biodegradable thermoplastics and elastomers. Arabidopsis thaliana plants that expressed a Pseudomonas aeruginosa PHA synthase that was modified for targeting to the **peroxisome** produced a PHA containing saturated and unsaturated 3-hydroxyalkanoic acids of 6-16 carbon atoms. Inclusions of PHA were found in glyoxysomes and leaf-type **peroxisomes** of dark-grown and light-grown plants, respectively, as well as in the vacuole.

3/3,AB/10 (Item 1 [redacted] file: 143)
DIALOG(R)File 143:Biol. & Agric. Index

1285857 H.W. WILSON RECORD NUMBER: BBAI00067263
Analysis of the alternative pathways for the b-oxidation of unsaturated fatty acids using transgenic plants synthesizing **polyhydroxyalkanoates** in **peroxisomes**
Allenbach, Laure
Poirier, Yves
Plant Physiology v. 124 no3 (Nov. 2000) p. 1159-68
DOCUMENT TYPE: Feature Article ISSN: 0032-0889

3/3,AB/11 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

15040713 PASCAL No.: 01-0198205
Production of polyesters in transgenic plants
Biopolyesters
POIRIER Yves
BABEL Wolfgang, ed; STEINBUECHEL Alexander, ed
Institut d'Ecologie-Biologie et Physiologie Vegetales, Universite de Lausanne, 1015 Lausanne, Switzerland
UFZ-Umweltforschungszentrum, Leipzig-Halle GmbH, Sektion Umweltmikrobiologie, Permoserstr. 15, 04318 Leipzig, Germany; Westf. Wilhelms-Universitaet, Muenster, Institut fuerMikrobiologi e, Correnssstr. 3 , 48148 Muenster, Germany
Journal: Advances in biochemical engineering, biotechnology, 2001, 71 209-240
Language: English
Polyhydroxyalkanoates (PHAs) are bacterial polyesters having the properties of biodegradable thermoplastics and elastomers. Synthesis of PHAs has been demonstrated in transgenic plants. Both polyhydroxybutyrate and the co-polymer poly(hydroxybutyrate-co-hydroxyvalerate) have been synthesized in the plastids of Arabidopsis thaliana and Brassica napus. Furthermore, a range of medium-chain-length PHAs has also been produced in plant **peroxisomes** . Development of agricultural crops to produce PHA on a large scale and at low cost will be a challenging task requiring a coordinated and stable expression of several genes. Novel extraction methods designed to maximize the use of harvested plants for PHA, oil, carbohydrate, and feed production will be needed. In addition to their use as plastics, PHAs can also be used to modify fiber properties in plants such as cotton. Furthermore, PHA can be exploited as a novel tool to study the carbon flux through various metabolic pathways, such as the fatty acid beta -oxidation cycle.

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Set	Items	Description
S1	1868	POLYHYDROXYALKANOATE?
S2	29	S1 AND PEROXISOME?
S3	11	RD (unique items)

? s s1 and fusion?

	1868	S1
	528486	FUSION?
S4	36	S1 AND FUSION?

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>>>Duplicate detection is not supported for File 306.

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>>>Record 266:264437 ignored; incomplete bibliographic data, not retained - in RD set
...completed examining records

S5	16	RD (unique items)

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>>>No matching display code(s) found in file(s): 65, 306

5/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11518644 21378195 PMID: 11485576

Matrix-assisted in vitro refolding of Pseudomonas aeruginosa class II **polyhydroxyalkanoate** synthase from inclusion bodies produced in recombinant Escherichia coli.

Rehm BH; Qi Q; Beermann BB; Hinz HJ; Steinbuchel A
Institut fur Mikrobiologie der Westfalischen Wilhelms-Universitat Munster, Corrensstrasse 3, 48149 Munster, Germany.

Biochemical journal (England) Aug 15 2001, 358 (Pt 1) p263-8, ISSN 0264-6021 Journal Code: 9YO
Languages: ENGLISH
Document type: Journal Article
Record type: In Process

In order to facilitate the large-scale preparation of active class II **polyhydroxyalkanoate** (PHA) synthase, we constructed a vector pT7-7 derivative that contains a modified phaC1 gene encoding a PHA synthase from Pseudomonas aeruginosa possessing six N-terminally fused histidine residues. Overexpression of this phaC1 gene under control of the strong O10 promoter was achieved in Escherichia coli BL21(DE3). The **fusion** protein was deposited as inactive inclusion bodies in recombinant E. coli, and contributed approx. 30% of total protein. The inclusion bodies were purified by selective solubilization, resulting in approx. 70-80% pure PHA synthase, then dissolved and denatured by 6 M guanidine hydrochloride. The denatured PHA synthase was reversibly immobilized on a Ni(2+)-nitrilotriacetate-agarose matrix. The matrix-bound **fusion** protein was refolded by gradual removal of the chaotropic reagent. This procedure avoided the aggregation of folding intermediates which often decreases the efficiency of refolding experiments. Finally, the refolded **fusion** protein was eluted with imidazole. The purified and refolded PHA synthase protein showed a specific enzyme activity of 10.8 m-units/mg employing (R/S)-3-hydroxydecanoyl-CoA as substrate, which corresponds to 27% of the maximum specific activity of the native enzyme. The refolding of

the enzyme was confirmed by CD spectroscopy. Deconvolution of the spectrum resulted in the following secondary structure prediction: 10% alpha-helix, 50% beta-sheet and 40% random coil. Gel filtration chromatography indicated an apparent molecular mass of 69 kDa for the refolded PHA synthase. However, light-scattering analysis of a 10-fold concentrated sample indicated a molecular mass of 128 kDa. These data suggest that the class II PHA synthase is present in an equilibrium of monomer and dimer.

5/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11383674 21311747 PMID: 11418562
Accumulation of the PhaP Phasin of Ralstonia eutropha Is Dependent on Production of Polyhydroxybutyrate in Cells.
York GM; Junker BH; Stubbe J; Sinskey AJ
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.
Journal of bacteriology (United States) Jul 2001, 183 (14) p4217-26, ISSN 0021-9193 Journal Code: HH3
Languages: ENGLISH
Document type: Journal Article
Record type: In Process
Polyhydroxyalkanoates (PHAs) are polyoxoesters that are produced by diverse bacteria and that accumulate as intracellular granules. Phasins are granule-associated proteins that accumulate to high levels in strains that are producing PHAs. The accumulation of phasins has been proposed to be dependent on PHA production, a model which is now rigorously tested for the phasin PhaP of Ralstonia eutropha. R. eutropha phaC PHA synthase and phaP phasin gene replacement strains were constructed. The strains were engineered to express heterologous and/or mutant PHA synthase alleles and a phaP-gfp translational **fusion** in place of the wild-type alleles of phaC and phaP. The strains were analyzed with respect to production of polyhydroxybutyrate (PHB), accumulation of PhaP, and expression of the phaP-gfp **fusion** . The results suggest that accumulation of PhaP is strictly dependent on the genetic capacity of strains to produce PHB, that PhaP accumulation is regulated at the level of both PhaP synthesis and PhaP degradation, and that, within mixed populations of cells, PhaP accumulation within cells of a given strain is not influenced by PHB production in cells of other strains. Interestingly, either the synthesis of PHB or the presence of relatively large amounts of PHB in cells (>50% of cell dry weight) is sufficient to enable PhaP synthesis. The results suggest that R. eutropha has evolved a regulatory mechanism that can detect the synthesis and presence of PHB in cells and that PhaP expression can be used as a marker for the production of PHB in individual cells.

5/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11124965 21167661 PMID: 11267773
Identification of the intracellular **polyhydroxyalkanoate** depolymerase gene of Paracoccus denitrificans and some properties of the gene product.
Gao D; Maehara A; Yamane T; Ueda S
FEMS microbiology letters (Netherlands) Mar 15 2001, 196 (2) p159-64, ISSN 0378-1097 Journal Code: FML
Languages: ENGLISH
Document type: Journal Article
Record type: In Process
Paracoccus denitrificans degraded poly(3-hydroxybutyrate) (PHB) in the cells under carbon source starvation. Intracellular poly(3-hydroxyalkanoate) (PHA) depolymerase gene (phaZ) was identified near the PHA synthase gene (phaC) of P. denitrificans. Cell extract of Escherichia coli carrying lacZ-phaZ **fusion** gene degraded

protease-treated PHB granules. Reaction products were thought to be mainly D(-)-3-hydroxybutyrate (3HB) dimer and [redacted]B oligomer. Diisopropylfluorophosphonate and Triton X-100 exhibited an inhibitory effect on the degradation of PHB granules. When cell extract of the recombinant E. coli was used, Mg(2+) ion inhibited PHB degradation. However, the inhibitory effect by Mg(2+) ion was not observed using the cell extract of P. denitrificans.

5/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10852390 20406520 PMID: 10952003
In vitro synthesis of poly(3-hydroxydecanoate): purification and enzymatic characterization of type II **polyhydroxyalkanoate** synthases PhaC1 and PhaC2 from Pseudomonas aeruginosa.
Qi Q; Steinbuchel A; Rehm BH
Institut fur Mikrobiologie, Westfalische Wilhelms-Universitat Munster, Germany.
Applied microbiology and biotechnology (GERMANY) Jul 2000, 54 (1) p37-43, ISSN 0175-7598 Journal Code: AMC
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
For the first time, the purification has been achieved of the type II **polyhydroxyalkanoate** (PHA) synthases PhaC1 and PhaC2 from Pseudomonas aeruginosa applying N-terminal His6-tag **fusions** and metal chelate affinity chromatography. In vivo His6-tagged PHA synthase activity was confirmed by functional expression of the corresponding genes in Escherichia coli, and PHA synthase activity could also be measured in vitro with the enzymes. The specific enzyme activity of PHA synthases PhaC1 and PhaC2 was 0.039 U mg(-1) and 0.035 U mg(-1) protein, respectively. Kinetic studies showed a lag phase for both PHA synthases using (R,S)-3-hydroxydecanoyl-CoA as substrate. Specific enzyme activity was increased to 0.055 U mg(-1) when the phasin GA24 from Ralstonia eutropha was added to the assay. CoA inhibited PHA synthase activity, and a Ki of 85 microM was determined. A two-enzyme system was established, employing commercially available acyl-CoA synthetase and PHA synthase, which allowed the in vitro de novo PHA granule formation and the in vitro synthesis of poly(3-hydroxydecanoate) exhibiting a weight average molar mass of 9.8 x 10(4) g mol(-1), and which occurred independently of pre-existing PHA granules.

5/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10758130 99102223 PMID: 9882674
Polyhydroxyalkanoate inclusion body-associated proteins and coding region in Bacillus megaterium.
McCool GJ; Cannon MC
Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003, USA.
Journal of bacteriology (UNITED STATES) Jan 1999, 181 (2) p585-92, ISSN 0021-9193 Journal Code: HH3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Polyhydroxyalkanoic acids (PHA) are carbon and energy storage polymers that accumulate in inclusion bodies in many bacteria and archaea in response to environmental conditions. This work presents the results of a study of PHA inclusion body-associated proteins and an analysis of their coding region in Bacillus megaterium 11561. A 7, 917-bp fragment of DNA was cloned and shown to carry a 4,104-bp cluster of 5 pha genes, phaP, -Q, -R, -B, and -C. The phaP and -Q genes were shown to be transcribed in one

orientation, each from a separate promoter, while immediately upstream, phaR, -B, and -C were divergently transcribed as a tricistronic operon. Transfer of this gene cluster to Escherichia coli and to a PhaC- mutant of Pseudomonas putida gave a Pha+ phenotype in both strains. Translational **fusions** to the green fluorescent protein localized PhaP and PhaC to the PHA inclusion bodies in living cells. The data presented are consistent with the hypothesis that the extremely hydrophilic protein PhaP is a storage protein and suggests that PHA inclusion bodies are not only a source of carbon, energy, and reducing equivalents but are also a source of amino acids.

5/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09996300 99069318 PMID: 9851987
Cloning and molecular analysis of the Poly(3-hydroxybutyrate) and Poly(3-hydroxybutyrate-co-3-hydroxyalkanoate) biosynthesis genes in Pseudomonas sp. strain 61-3.
Matsusaki H; Manji S; Taguchi K; Kato M; Fukui T; Doi Y
Polymer Chemistry Laboratory and the RIKEN Group of Japan Science and Technology Corporation, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan.
Journal of bacteriology (UNITED STATES) Dec 1998, 180 (24) p6459-67, ISSN 0021-9193 Journal Code: HH3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Two types of **polyhydroxyalkanoate** (PHA) biosynthesis gene loci (phb and pha) of Pseudomonas sp. strain 61-3, which produces a blend of poly(3-hydroxybutyrate) [P(3HB)] homopolymer and a random copolymer poly(3-hydroxybutyrate-co-3-hydroxyalkanoate) [P(3HB-co-3HA] consisting of 3HA units of 4 to 12 carbon atoms, were cloned and analyzed at the molecular level. In the phb locus, three open reading frames encoding polyhydroxybutyrate (PHB) synthase (PhbCPs), beta-ketothiolase (PhbAPs), and NADPH-dependent acetoacetyl coenzyme A reductase (PhbBPs) were found. The genetic organization showed a putative promoter region, followed by phbBPs-phbAPs-phbCPs. Upstream from phbBPs was found the phbRPs gene, which exhibits significant similarity to members of the AraC/XylS family of transcriptional activators. The phbRPs gene was found to be transcribed in the opposite direction from the three structural genes. Cloning of phbRPs in a relatively high-copy vector in Pseudomonas sp. strain 61-3 elevated the levels of beta-galactosidase activity from a transcriptional phb promoter-lacZ **fusion** and also enhanced the 3HB fraction in the polyesters synthesized by this strain, suggesting that PhbRPs is a positive regulatory protein controlling the transcription of phbBACPs in this bacterium. In the pha locus, two genes encoding PHA synthases (PhaC1Ps and PhaC2Ps) were flanked by a PHA depolymerase gene (phaZPs), and two adjacent open reading frames (ORF1 and phaDPs), and the gene order was ORF1, phaC1Ps, phaZPs, phaC2Ps, and phaDPs. Heterologous expression of the cloned fragments in PHA-negative mutants of Pseudomonas putida and Ralstonia eutropha revealed that PHB synthase and two PHA synthases of Pseudomonas sp. strain 61-3 were specific for short chain length and both short and medium chain length 3HA units, respectively.

5/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09569364 97394927 PMID: 9251189
Polyhydroxyalkanoate production in Rhodobacter capsulatus: genes, mutants, expression, and physiology.
Kranz RG; Gabbert KK; Locke TA; Madigan MT
Department of Biology, Washington University, St. Louis, Missouri 63130, USA. kranz@wustlb.wustlb.edu

Applied and environmental microbiology (UNITED STATES) Aug 1997, 63 (8) p3003-9, ISSN 0099-2240 Journal Code: 6K6

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Like many other prokaryotes, the photosynthetic bacterium Rhodobacter capsulatus produces high levels of **polyhydroxyalkanoates** (PHAs) when a suitable carbon source is available. The three genes that are traditionally considered to be necessary in the PHA biosynthetic pathway, phaA (beta-ketothiolase), phaB (acetoacetylcoenzyme A reductase), and phaC (PHA synthase), were cloned from Rhodobacter capsulatus. In R. capsulatus, the phaAB genes are not linked to the phaC gene. Translational beta-galactosidase **fusions** to phaA and phaC were constructed and recombined into the chromosome. Both phaC and phaA were constitutively expressed regardless of whether PHA production was induced, suggesting that control is posttranslational at the enzymatic level. Consistent with this conclusion, it was shown that the R. capsulatus transcriptional nitrogen-sensing circuits were not involved in PHA synthesis. The doubling times of R. capsulatus transcriptional nitrogen-sensing circuits were not involved in PHA synthesis. The doubling times of R. capsulatus grown on numerous carbon sources were determined, indicating that this bacterium grows on C2 to C12 fatty acids. Grown on acetone, caproate, or heptanoate, wild-type R. capsulatus produced high levels of PHAs. Although a phaC deletion strain was unable to synthesize PHAs on any carbon source, phaA and phaAB deletion strains were able to produce PHAs, indicating that alternative routes for the synthesis of substrates for the synthase are present. The nutritional versatility and bioenergetic versatility of R. capsulatus, coupled with its ability to produce large amounts of PHAs and its genetic tractability, make it an attractive model for the study of PHA production.

5/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09352034 97313874 PMID: 9170275

Functional expression of the PHA synthase gene phaC1 from Pseudomonas aeruginosa in Escherichia coli results in poly(3-hydroxyalkanoate) synthesis.

Langenbach S; Rehm BH; Steinbuchel A

Institut fur Mikrobiologie, Westfalische Wilhelmus-Universitt Munster, Germany.

FEMS microbiology letters (NETHERLANDS) May 15 1997, 150 (2) p303-9, ISSN 0378-1097 Journal Code: FML

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The potential of the production of **polyhydroxyalkanoates** (PHA), consisting of medium-chain-length (MCL) hydroxyfatty acids (C5-C14), in recombinant Escherichia coli was investigated. E. coli mutants affected in fatty acid degradation and fatty acid de novo synthesis were employed. We established the functional expression of the Pseudomonas aeruginosa PHA synthase gene phaC1. The coding region of phaC1 was subcloned via PCR into vector pBluescript SK-. The resulting plasmid pBHR71 enabled functional expression of phaC1 under lac promoter control and conferred synthesis and accumulation of PHA to various strains of E. coli. PHA synthesis was analysed with respect to the carbon source in various E. coli fad and fab mutants. This study provided evidence that intermediates of the fatty acid beta-oxidation can be directed to PHA synthesis and that 3-hydroxydecanoyl-CoA is the main substrate for PHA synthase PhaC1 from P. aeruginosa. The E. coli fadB mutant LS1298 containing plasmid pBHR71 and cultivated in LB medium containing 0.5% (w/v) decanoate revealed the strongest accumulation of PHA contributing to about 21% of the cellular dry weight, which was composed of 2.5 mol% 3-hydroxyhexanoate, 20 mol% 3-hydroxyoctanoate, 72.5 mol% 3-hydroxydecanoate and 5 mol%

3-hydroxydodecanoate.

5/3,AB/9 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07748405 Genuine Article#: 204DU Number of References: 39
Title: Substrate and binding specificities of bacterial polyhydroxybutyrate depolymerases (ABSTRACT AVAILABLE)
Author(s): Kasuya K; Ohura T; Masuda K; Doi Y (REPRINT)
Corporate Source: INST PHYS & CHEM RES,RIKEN, POLYMER CHEM LAB, 2-1 HIROSAWA/WAKO/SAITAMA 3510198/JAPAN/ (REPRINT); INST PHYS & CHEM RES,RIKEN, POLYMER CHEM LAB/WAKO/SAITAMA 3510198/JAPAN/; SAITAMA UNIV,GRAD SCH SCI & ENGN/URAWA/SAITAMA 3380825/JAPAN/
Journal: INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, 1999, V24, N4 (MAY), P329-336
ISSN: 0141-8130 Publication date: 19990500
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: The substrate specificities of three extracellular polyhydroxybutyrate (PHB) depolymerases from Alcaligenes faecalis (PhaZ(Afa)), Pseudomonas stutzeri (PhaZ(Pst)), and Comamonas acidovorans (PhaZ(Cac)), which are grouped into types A and B based on the position of a lipase box sequence in the catalytic domain, were examined for films of 12 different aliphatic polyesters. Each of these PHB depolymerases used was capable of hydrolyzing poly(3-hydroxybutyrate) (P(3HB)), poly(3-hydroxypropionate) (P(3HP)), poly(4-hydroxybutyrate) (P(4HB)), poly(ethylene succinate) (PESU), and poly(ethylene adipate) (PEA) but could not hydrolyze another seven polyesters. In addition, the binding characteristics of substrate binding domains from PhaZ(Afa), PhaZ(Cac), and PHB depolymerase from Comamonas testosteroni (PhaZ(Cte)) were studied by using **fusions** with glutathione S-transferase (GST). All of **fusion** proteins adsorbed strongly on the surfaces of polyester granules of P(3HB), P(3HP), and poly(2-hydroxypropionate) (P(2HP)) which was not hydrolyzed by the PHB depolymerases used in this study, while they did not bind on Avicel and chitin granules. The adsorption kinetics of the **fusion** proteins to the surface of P(3HB) and P(2HP) granules were found to obey the Langmuir isotherm. The cross-area per molecule of **fusion** protein bound to P(3HB) granules was estimated to be 12 +/- 4 nm(2)/molecule. It has been suggested that the active sites in catalytic domains of PHB depolymerases have a similar conformational structure, and that several amino acids in substrate-binding domains of PHB depolymerases interact specifically with the surface of polyesters.

5/3,AB/10 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07637140 Genuine Article#: 190FR Number of References: 28
Title: Effect of sterilization on the physical and structural characteristics of polyhydroxyoctanoate (PHO) (ABSTRACT AVAILABLE)
Author(s): Marois Y; Zhang Z; Vert M; Deng XY; Lenz R; Guidoin R (REPRINT)

Corporate Source: UNIV LAVAL,EXPT SURG LAB, ROOM 1701, SERV BLDG/QUEBEC CITY/PQ G1K 7P4/CANADA/ (REPRINT); UNIV LAVAL,EXPT SURG LAB/QUEBEC CITY/PQ G1K 7P4/CANADA/; UNIV MONTPELLIER 1,FAC PHARM, CRBA, CNRS, URA 1465/MONTPELLIER//FRANCE/; CHUQ,CTR RECH, BIOMAT INST/QUEBEC CITY/PQ/CANADA/; UNIV MASSACHUSETTS,/AMHERST//MA/01003
Journal: JOURNAL OF BIOMATERIALS SCIENCE-POLYMER EDITION, 1999, V10, N4, P 469-482
ISSN: 0920-5063 Publication date: 19990000

Publisher: VSP BV, PO BOX 346, 3700 AH ZEIST, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: The present study examined the potential applicability of poly(S-hydroxy octanoate) (PHO), a bacterial polyester, as a candidate for biomaterial applications, by investigating the effect of sterilization on the physical and structural characteristics of PHO. PHO-cast films were sterilized by either ethylene oxide (EO) gas at 38 degrees C or gamma radiation (2.5 Mrad) in air at room temperature. The physical characteristics of the EO and gamma-sterilized PHO were determined by scanning electron microscopy (SEM) and tensile strength analyses. In addition, various analytical methods were used to detect modifications in the chemical and morphological structure of PHO, namely, electron spectroscopy for chemical analysis (ESCA), Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), wide angle X-ray diffraction (WAXD), and size exclusion chromatography (SEC). The results show that EO sterilization did not modify the chemical and physical characteristics of PHO, however, significant modifications in both the structural and tensile properties were observed with gamma-sterilized PHO. These changes accounted for decreases in both the weight average, number average and melting temperature, and increases in the heat of **fusion** and tensile strength. No residual EO was detected following sterilization as revealed by head-space chromatography. The physical and structural properties of PHO were shown to be well preserved following EO sterilization, whereas gamma radiation caused random chain scission and physical cross-linking, a frequent phenomenon observed with organic polymers.

5/3,AB/11 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06819590 Genuine Article#: ZU798 Number of References: 34
Title: Radiation crosslinking of a bacterial medium-chain-length poly(hydroxyalkanoate) elastomer from tallow (ABSTRACT AVAILABLE)
Author(s): Ashby RD (REPRINT) ; Cromwick AM; Foglia TA
Corporate Source: USDA ARS,EASTERN REG RES CTR, 600 E MERMAID LANE/WYNDMOOR//PA/19038 (REPRINT)
Journal: INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, 1998, V23, N1 (JUL), P61-72
ISSN: 0141-8130 Publication date: 19980700
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: Pseudomonas resinovorans produces a medium-chain-length poly(hydroxyalkanoate) (MCL-PHA) copolymer when grown on tallow (PHA-tal). This polymer had a repeat unit composition ranging from C4 to C14 with some mono-unsaturation in the C12 and C14 alkyl side chains. Thermal analysis indicated that the polymer;was semi-crystalline with a melting temperature (T-m) of 43.5 +/- 0.2 degrees C and a glass transition temperature (T-g) of -43.4 +/- 2.0 degrees C. The presence of unsaturated side chains allowed crosslinking by gamma-irradiation. Irradiated polymer films had decreased solubility in organic solvents that indicated an increase in the crosslink density within the film matrix. The addition of linseed oil to the gamma-irradiated film matrix enhanced polymer recovery while minimizing chain scission. Linseed oil also caused a decrease in the enthalpy of **fusion** (Delta H-m) of the films (by an average of 60%) as well as enhanced mineralization. The effects of crosslinking on the mechanical properties and biodegradability of the polymer were determined. Radiation had no effect on the storage modulus (E') of the polymer. However, radiation doses of 25 and 50 kGy did increase the Young modulus of the polymer by 129 and 114%, and the tensile strength of the polymer by 76 and 35%, respectively. Finally, the formation of a higher crosslink density within the polymer matrix decreased the

biodegradability of t[redacted] PHA films. (C) 1998 Elsevier Science B.V. All rights reserved.

5/3,AB/12 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06341053 Genuine Article#: YK709 Number of References: 49
Title: Biochemical and molecular characterization of the polyhydroxybutyrate depolymerase of Comamonas acidovorans YM1609, isolated from freshwater (ABSTRACT AVAILABLE)
Author(s): Kasuya KI; Inoue Y; Tanaka T; Akehata T; Iwata T; Fukui T; Doi Y (REPRINT)
Corporate Source: INST PHYS & CHEM RES,POLYMER CHEM LAB, 2-1 HIROSAWA/WAKO/SAITAMA 35101/JAPAN/ (REPRINT); INST PHYS & CHEM RES,POLYMER CHEM LAB/WAKO/SAITAMA 35101/JAPAN/; TOKYO INST TECHNOL,FAC BIOSCI & BIOTECHNOL, DEPT BIOENGN/YOKOHAMA/KANAGAWA 227/JAPAN/; SCI UNIV TOKYO,FAC ENGN, DEPT IND CHEM, SHINJYUKU KU/TOKYO 162//JAPAN/
Journal: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 1997, V63, N12 (DEC), P 4844-4852
ISSN: 0099-2240 Publication date: 19971200
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE
Abstract: Comamonas acidovorans YM1609 secreted a polyhydroxybutyrate (PHB) depolymerase into the culture supernatant when it was cultivated on poly(3-hydroxybutyrate) [P(3HB)] or poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] as the sole carbon source. The PHB depolymerase was purified from culture supernatant of C. acidovorans by two chromatographic methods, and its molecular mass was determined as 45,000 Da by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The enzyme was stable at temperatures below 37 degrees C and at pH values of 6 to 10, and its activity was inhibited by diisopropyl fluorophosphonate. The liquid chromatography analysis of water-soluble products revealed that the primary product of enzymatic hydrolysis of P(3HB) was a dimer of 3-hydroxybutyric acid. Kinetics of enzymatic hydrolysis of P(3HB) film were studied. In addition, a gene encoding the PHB depolymerase was cloned from the C. acidovorans genomic library. The nucleotide sequence of this gene was found to encode a protein of 494 amino acids (M-r, 51,018 Da). Furthermore, by analysis of the N-terminal amino acid sequence of the purified enzyme, the molecular mass of the mature enzyme was calculated to be 48,628 Da. Analysis of the deduced amino acid sequence suggested a domain structure of the protein containing a catalytic domain, fibronectin type III module as linker, and a putative substrate-binding domain. Electron microscopic visualization of the mixture of P(3HB) single crystals and a **fusion** protein of putative substrate-binding domain with glutathione S-transferase demonstrated that the **fusion** protein adsorbed strongly and homogeneously to the surfaces of P(3HB) single crystals.

5/3,AB/13 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04923461 Genuine Article#: UT135 Number of References: 42
Title: MICROBIAL SYNTHESIS OF POLY(BETA-HYDROXYALKANOATES) CONTAINING FLUORINATED SIDE-CHAIN SUBSTITUENTS (Abstract Available)
Author(s): KIM O; GROSS RA; HAMMAR WJ; NEWMARK RA
Corporate Source: UNIV MASSACHUSETTS,DEPT CHEM,1 UNIV AVE/LOWELL//MA/01854; UNIV MASSACHUSETTS,DEPT CHEM/LOWELL//MA/01854; 3M CO,LIFE SCI RES LAB/ST PAUL//MN/55144
Journal: MACROMOLECULES, 1996, V29, N13 (JUN 17), P4572-4581

ISSN: 0024-9297
Language: ENGLISH Document Type: ARTICLE
Abstract: The preparation of novel fluorinated poly(beta-hydroxyalkanoates), PHAs, was carried out using Pseudomonas oleovorans (ATCC 29347) and Pseudomonas putida (KT 2442) as biocatalysts. These organisms were first grown on 40 mM sodium citrate prior to studying polymer formation in the second stage using 1:1 molar mixtures of nonanoic acid (NA) and fluorinated acid cosubstrates. The following fluoro acids were synthesized and used in this study: 6,6,6-trifluorohexanoic acid (TFHxA), 6,6,7,7,8,8,8-heptafluorooctanoic acid (HpFOA), 6,6,7,7,8,8,9,9,9-nonafluorononanoic acid (INFNA), and 6,6,7,7,8,8,9,9, 10,10,11,11,11-tridecafluoroundecanoic acid (TDFUDA). In general, the use of NA/fluoro acid cosubstrate mixtures instead of only NA in second-stage cultivations resulted in little to no cellular toxicity as measured by values of colony-forming units per milliliter. The mol percent incorporations of fluorinated side chains was determined by H-1 and F-19 NMR spectroscopies, and peak assignments were made using two-dimensional reverse-detected heteronuclear multiplet quantum correlation (HMQC) as well as H-1-H-1 correlation spectroscopy (COSY). P. putida formed PHA after a 3-day second-stage cultivation time with 17.3 mol % fluorinated side chains using NA/NFNA as cosubstrates. For shorter second-stage cultivation times (1 day) where product yields were relatively higher, 0.3 g/L of product was formed that contained 6.4 mol % fluoroalkanoate side groups using P. oleovorans as the biocatalyst and NA/HpFOA as cosubstrates. The incorporation of 12.4 mol % fluoroalkanoate repeat units resulted in products which showed melting at higher temperatures (55-80 degrees C), crystallized at faster rates from the melt, and had higher heats of **fusion**. Investigation of the surface free energy of products by surface contact angle measurements showed only a modest increase from 87 to 94 degrees for PHAs containing 0 and 17.3 mol % fluorinated side chains.

5/3,AB/14 (Item 1 from file: 94)
DIALOG(R)File 94:JICST-EPlus

04521996 JICST ACCESSION NUMBER: 00A0090031 FILE SEGMENT: JICST-E
Bacterial **Polyhydroxyalkanoates**(PHAs) from Palm Oil, Palm Kernel Oil and their Derivatives.
TAN I K P (1); KUMAR K S (1); THEANMALAR M (1); GAN S-N (1)
(1) Univ. Malaya, Kuala Lumpur, Mys
Biotechnol Sustain Util Biol Resour Trop Vol 11, 1996, PAGE.303-305, TBL.1, REF.3
JOURNAL NUMBER: K19990497E
UNIVERSAL DECIMAL CLASSIFICATION: 663.16+663.18
LANGUAGE: English COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Conference Proceeding
ARTICLE TYPE: Commentary
MEDIA TYPE: Printed Publication

5/3,AB/15 (Item 1 from file: 99)
DIALOG(R)File 99:Wilson Appl. Sci & Tech Abs

2287782 H.W. WILSON RECORD NUMBER: BAST00076849
Targeted, PCR-based gene disruption in cyanobacteria: inactivation of the polyhydroxyalkanoic acid synthase genes in Synechocystis sp. PCC6803
Taroncher-Oldenburg, G; Stephanopoulos, G
Applied Microbiology and Biotechnology v. 54 no5 (Nov. 2000) p. 677-80
DOCUMENT TYPE: Feature Article ISSN: 0175-7598

ABSTRACT: The authors outline a polymerase chain reaction-based method for

the efficient construction of targeted gene disruptions and gene **fusions** in the cyanobacterium Synechocystis sp. PCC6803. A gene conversion cassette was synthesized that targeted the polyhydroxyalkanoic acid (PHA) synthase genes. PHA production in Synechocystis, under normal and high production culture conditions, was not detectable after transformation. The applicability of this method to the genetic inactivation of the phaE-CSyn gene cluster demonstrated its potential for genetically engineering cyanobacteria and investigating functional genomics in Synechocystis..

5/3,AB/16 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00165066
IDENTIFYING NO.: 9905419 AGENCY CODE: NSF
Biogenesis of **Polyhydroxyalkanoate** Inclusion Bodies in Bacillus Megaterium
PRINCIPAL INVESTIGATOR: Cannon, Maura C
PERFORMING ORG.: University of Massachusetts Amherst, Dept. of Biochemistry&Molecular Biology, Amherst, MA 01003
PROJECT MONITOR: Harriman, Philip
SPONSORING ORG.: National Science Foundation, MCB, 4201 Wilson Boulevard , Arlington, Virginia 22230
DATES: 20000215 TO 20020131 FY : 2000 FUNDS: $200,046 (200000)
SUMMARY: The storage polymer polyhydroxyalkanoic acid (PHA) accumulates in inclusion bodies in most bacteria and archaea. Given the widespread occurrence and influence on the metabolism and physiology of the cell of these inclusion bodies, it is surprising that so little is known about their structure and biogenesis. This project will identify the functions of small proteins (pha proteins) that co-purify with PHA inclusion bodies. The focus is on localizing these proteins throughout growth, and on determining the precise amino acid sequences required for accurate localization to take place, using Bacillus megaterium. Six pha genes at two loci have been identified in B. megaterium. The functions of three of these genes (phaA, B and C) can be predicted based on homology to known genes, while those of the remaining three genes (phaP, Q and R) are unknown. Intracellular localization of phaQ and phaR will be examined using translational **fusions** to the green fluorescent protein (GFP). The identity of the amino acid sequences required for localization of phaP and phaC (and phaQ and phaR, if appropriate) to the inclusion bodies will be determined. The phenotypes of phaP, Q and R deletion mutants will be characterized, and the interdependence of these proteins on each other for accurate localization will be tested by analyzing subcellular localization of each of the pha proteins in various deletion mutants. The data from these experiments should indicate the extent to which phaP, Q and R are involved in the assembly of PHA inclusion bodies, and how they interact with each other in

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peroxisom? and target? and fusion?

          41153  PEROXISOM?
         997835  TARGET?
         528486  FUSION?
      S6     371  PEROXISOM? AND TARGET? AND FUSION?
? s s6 and pha

            371  S6
          45772  PHA
      S7      0  S6 AND PHA
? ds


Set     Items   Description
S1       1868   POLYHYDROXYALKANOATE?
S2         29   S1 AND PEROXISOME?
S3         11   RD (unique items)
S4         36   S1 AND FUSION?
S5         16   RD (unique items)
S6        371   PEROXISOM? AND TARGET? AND FUSION?
S7          0   S6 AND PHA
? s pha and peroxisom?

          45772  PHA
          41153  PEROXISOM?
      S8     31  PHA AND PEROXISOM?
? s s8 not s3

             31  S8
             11  S3
      S9     23  S8 NOT S3
? rd

>>>Duplicate detection is not supported for File 306.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
     S10     14  RD (unique items)
? t s10/3,ab/all

>>>No matching display code(s) found in file(s): 65, 306

 10/3,AB/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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13059536   BIOSIS NO.: 200100266685
Transgenic tobacco producing co-polymer in peroxisomes.
AUTHOR: Arai Yuko; Nakashita Hideo; Kobayashi Yumiko; Suzuki Yoshikatsu;
  Doi Yoshiharu; Yamaguchi Isamu
JOURNAL: Plant and Cell Physiology  42 (Supplement):ps203 2001
MEDIUM: print
CONFERENCE/MEETING: Symposia and Workshops of the 2001 Annual Meeting of
the Japanese Society of Plant Physiologists  Fukuoka, Japan  March 23-26,
2001
SPONSOR: Japanese Society of Plant Physiologists
ISSN: 0032-0781
RECORD TYPE: Citation
```

LANGUAGE: English
SUMMARY LANGUAGE: Engli
2001

10/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12325315 BIOSIS NO.: 200000078817
Increased flow of fatty acids toward beta-oxidation in developing seeds of Arabidopsis deficient in diacylglycerol acyltransferase activity or synthesizing medium-chain-length fatty acids.
AUTHOR: Poirier Yves(a); Ventre Giovanni; Caldelari Daniela
AUTHOR ADDRESS: (a)Institut d'Ecologie-Biologie et Physiologie Vegetales, Batiment de Biologie, Universite de Lausanne, CH-1015, Lausanne** Switzerland
JOURNAL: Plant Physiology (Rockville) 121 (4):p1359-1366 Dec., 1999
ISSN: 0032-0889
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Synthesis of polyhydroxylakanoates (PHAs) from intermediates of fatty acid beta-oxidation was used as a tool to study fatty acid degradation in developing seeds of Arabidopsis. Transgenic plants expressing a **peroxisomal PHA** synthase under the control of a napin promoter accumulated **PHA** in developing seeds to a final level of 0.06 mg g-1 dry weight. In plants co-expressing a plastidial acylacyl carrier protein thioesterase from Cuphea lanceolata and a **peroxisomal PHA** synthase, approximately 18-fold more **PHA** accumulated in developing seeds. The proportion of 3-hydroxydecanoic acid monomer in the **PHA** was strongly increased, indicating a large flow of capric acid toward beta-oxidation. Furthermore, expression of the **peroxisomal PHA** synthase in an Arabidopsis mutant deficient in the enzyme diacylglycerol acyltransferase resulted in a 10-fold increase in **PHA** accumulation in developing seeds. These data indicate that plants can respond to the inadequate incorporation of fatty acids into triacylglycerides by recycling the fatty acids via beta-oxidation and that a considerable flow toward beta-oxidation can occur even in a plant tissue primarily devoted to the accumulation of storage lipids.

1999

10/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12266907 BIOSIS NO.: 200000020409
Polyhydroxyalkanoate synthesis in transgenic plants as a new tool to study carbon flow through beta-oxidation.
AUTHOR: Mittendorf Volker; Bongcam Vanessa; Allenbach Laure; Coullerez Geraldine; Martini Nobert; Poirier Yves(a)
AUTHOR ADDRESS: (a)Institut d'Ecologie-Biologie et Physiologie Vegetales, Universite de Lausanne, CH-1015, Lausanne**Switzerland
JOURNAL: Plant Journal 20 (1):p45-55 Oct., 1999
ISSN: 0960-7412
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Transgenic plants producing **peroxisomal** polyhydroxyalkanoate (**PHA**) from intermediates of fatty acid degradation were used to study carbon flow through the beta-oxidation cycle. Growth of transgenic plants in media containing fatty acids conjugated to Tween detergents resulted in an increased accumulation of **PHA** and incorporation into the polyester of monomers derived from the beta-oxidation of these fatty acids. Tween-laurate was a stronger inducer of beta-oxidation, as measured by acyl-CoA oxidase activity, and a more potent modulator of **PHA** quantity and monomer composition than Tween-oleate. Plants co-expressing a **peroxisomal PHA** synthase with a capryl-acyl carrier protein thioesterase from Cuphea lanceolata produced eightfold more **PHA** compared to plants expressing only the **PHA** synthase. **PHA** produced in double transgenic plants contained mainly saturated monomers ranging from 6 to 10 carbons, indicating an enhanced flow of capric acid towards beta-oxidation. Together, these results support the hypothesis that plant cells have mechanisms which sense levels of free or esterified unusual fatty acids, resulting in changes in the activity of the beta-oxidation cycle as well as removal and degradation of these unusual fatty acids through beta-oxidation. Such enhanced flow of fatty acids through beta-oxidation can be utilized to modulate the amount and composition of **PHA** produced in transgenic plants. Furthermore, synthesis of PHAs in plants can be used as a new tool to study the quality and relative quantity of the carbon flow through beta-oxidation as well as to analyse the degradation pathway of unusual fatty acids.

1999

10/3,AB/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07223155 Genuine Article#: 138WU Number of References: 34
Title: Synthesis of medium-chain-length polyhydroxyalkanoates in Arabidopsis thaliana using intermediates of **peroxisomal** fatty acid beta-oxidation (ABSTRACT AVAILABLE)
Author(s): Mittendorf V; Robertson EJ; Leech RM; Kruger N; Steinbuchel A; Poirier Y (REPRINT)
Corporate Source: UNIV LAUSANNE,INST BIOL & PHYSIOL VEGETALES, BATIMENT BIOL/CH-1015 LAUSANNE//SWITZERLAND/ (REPRINT); UNIV LAUSANNE,INST BIOL & PHYSIOL VEGETALES/CH-1015 LAUSANNE//SWITZERLAND/; UNIV YORK,DEPT BIOL/YORK YO1 5DD/N YORKSHIRE/ENGLAND/; UNIV MUNSTER,INST MIKROBIOL/D-48149 MUNSTER//GERMANY/
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1998, V95, N23 (NOV 10), P13397-13402
ISSN: 0027-8424 Publication date: 19981110
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: Polyhydroxyalkanoate (**PHA**) is a family of polymers composed primarily of R-3-hydroxyalkanoic acids, These polymers have properties of biodegradable thermoplastics and elastomers. Medium chain-length PHAs (MCL-PHAs) are synthesized in bacteria by using intermediates of the beta-oxidation of alkanoic acids, To assess the feasibility of producing MCL-PHAs in plants, Arabidopsis thaliana mas transformed with the PhaC1 synthase from Pseudomonas aeruginosa modified for **peroxisome** targeting by addition of the carboxyl 34 amino acids from the Brassica napus isocitrate lyase, Immunocytochemistry demonstrated that the modified **PHA** synthase was appropriately targeted to leaf-type **peroxisomes** in light-grown plants and glyoxysomes in dark-grown plants. Plants expressing the **PHA** synthase accumulated electron-lucent inclusions in the glyoxysomes and leaf-type **peroxisomes**, as well as in the vacuole, These

inclusions were similar to bacterial **PHA** inclusions. Analysis of plant extracts by GC and mass spectrometry demonstrated the presence of MCL-**PHA** in transgenic plants to approximately 1 mg per g of dry weight. The plant **PHA** contained saturated and unsaturated 3-hydroxyalkanoic acids ranging from six to 16 carbons with 41% of the monomers being 3-hydroxyoctanoic acid and 3-hydroxyoctenoic acid, These results indicate that the beta-oxidation of plant fatty acids can generate a broad range of R-3-hydroxyacyl-CoA intermediates that can be used to synthesize MCL-PHAs.

10/3,AB/5 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04277914 Genuine Article#: RU212 Number of References: 68
Title: IDENTIFICATION OF **PEROXISOMAL** MEMBRANE GHOSTS WITH AN EPITOPE-TAGGED INTEGRAL MEMBRANE-PROTEIN IN YEAST MUTANTS LACKING **PEROXISOMES** (Abstract Available)
Author(s): PURDUE PE; LAZAROW PB
Corporate Source: CUNY MT SINAI SCH MED,DEPT CELL BIOL & ANAT/NEWYORK//NY/10029
Journal: YEAST, 1995, V11, N11 (SEP 15), P1045-1060
ISSN: 0749-503X
Language: ENGLISH Document Type: ARTICLE
Abstract: Many yeast **peroxisome** biogenesis mutants have been isolated in which **peroxisomes** appear to be completely absent. Introduction of a wild-type copy of the defective gene causes the reappearance of **peroxisomes**, despite the fact that new **peroxisomes** are thought to form only from pre-existing **peroxisomes**. This apparent paradox has been explained for similar human mutant cell lines (from patients with Zellweger syndrome) by the discovery of **peroxisomal** membrane ghosts in the mutant cells (Santos, M. J., T. Imanaka, H. Shio, G. M. Small and P. B. Lazarow. 1988. Science 239, 1536-1538). Introduction of a wild-type gene is thought to restore to the ghosts the ability to import matrix proteins, and thus lead to the refilling of the **peroxisomes**. It is vitally important to our understanding of **peroxisome** biogenesis to determine whether the yeast mutants contain ghosts. We have solved this problem by introducing an epitope-tagged version of Pas3p, a **peroxisome** integral membrane protein (that is essential for **peroxisome** biogenesis). Nucleotides encoding a nine amino acid HA epitope were added to the PAS3 gene immediately before the stop codon. The tagged gene (PAS3(HA)) was inserted in the genome, replacing the wild-type gene at its normal locus. It was fully functional (the cells assembled **peroxisomes** normally and grew on oleic acid) but the expression level was too low to detect the protein with monoclonal antibody 12CA5. PAS3(HA), was expressed in greater quantity from an episomal plasmid with the CUP1 promoter. The gene product, Pas3(**PHA**), was detected by immunogold labelling on the membranes of individual and clustered **peroxisomes**; the dusters appeared as large spots in immunofluorescence. PAS3(HA) was similarly expressed in **peroxisome** biogenesis mutants peb2 and peb4, which lack morphologically recognizable **peroxisomes**. Gold-labelled membranes were clearly visible in both mutants: in peb2 the labelled membrane vesicles were generally much smaller than those in peb4, which resembled normal **peroxisomes** in size.

10/3,AB/6 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

03098462 Genuine Article#: NE656 Number of References: 48
Title: DIETARY SUPPLEMENTATION WITH VERY LONG-CHAIN N-3 FATTY-ACIDS IN MAN

DECREASES EXPRESSION OF THE INTERLEUKIN-2 RECEPTOR (CD25) ON MITOGEN-STIMULATED LYMPHOCYTES FROM PATIENTS WITH INFLAMMATORY SKIN DISEASES (Abstract Available)
Author(s): SOYLAND E; LEA T; SANDSTAD B; DREVON A
Corporate Source: UNIV OSLO,INST NUTR RES,DIETARY RES SECT,POB 1117/N-0317 OSLO//NORWAY/; AKER UNIV HOSP,RIKSHOSP,INST GEN RHEUMATOL & IMMUNOL/OSLO//NORWAY/
Journal: EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, 1994, V24, N4 (APR), P 236-242
ISSN: 0014-2972
Language: ENGLISH Document Type: ARTICLE
Abstract: T-cell activation and cytokine production play an important role in several chronic inflammatory diseases. Because n-3 fatty acids exert beneficial effects on the clinical state of some of these diseases, we examined the effect of dietary supplementation of n-3 fatty acids on T-cell proliferation, expression of CD25 (interleukin-2 receptor alpha-chain), secretion of interleukin-2, interleukin-6 and tumour necrosis factor from T-cells from patients with psoriasis and atopic dermatitis.

During 4 months, 21 patients supplied 6 g of highly concentrated ethyl esters of EPA and DHA in gelatin capsules daily to their diet. In the control group 20 patients supplied 6 g per day of corn oil in gelatin capsules to their diet.

Eicosapentaenoic acid (20:5, n-3) of serum phospholipids increased from 14 (min 4-max 42) to 81 (min 59-max 144) mg l(-1) ($P < 0.01$) in patients with atopic dermatitis receiving n-3 fatty acids, and from 25 (min 7-max 66) to 74 (min 46-max 142) mg l(-1) ($P < 0.01$) in patients with psoriasis, whereas docosahexaenoic acid (22:6, n-3) increased from 65 (min 46-max 120) to 92 (min 54-max 121) mg l(-1) ($P < 0.05$) and from 81 (min 38-max 122) to 92 (min 63-max 169) mg l(-1) (NS) in atopic and psoriatic patients, respectively. The changes in the serum phospholipid fatty acid profile in the groups receiving n-3 fatty acids, correlate to the dietary intake of corresponding fatty acids. There was no significant change in the fatty acid pattern of serum phospholipids in the corn oil group before and after supplementation.

Mitogen-induced secretion of interleukin-6 was significantly higher in patients with psoriasis compared to patients with atopic dermatitis, whereas the secretion of interleukin-2, tumour necrosis factor, **PHA**-induced T-cell proliferation and expression of CD25 on lymphocytes were similar in the two groups of patients. Patients receiving supplementation of n-3 fatty acids decreased significantly the percentage of CD25 positive lymphocytes from 40.5 before start to 35.5 ($P < 0.05$) after the trial. The patients who received corn oil increased the level of tumour necrosis factor from 10.95 pg ml(-1) before start to 1536 pg ml(-1) after the trial ($P < 0.05$). In conclusion, dietary intake of very long-chain n-3 fatty acids may suppress the expression of CD25 positive lymphocytes, which may partly account for the anti-inflammatory effect exerted by these fatty acids.

10/3,AB/7 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02571818 Genuine Article#: LM409 Number of References: 5
Title: **PEROXISOMAL** DISORDERS - CONCENTRATIONS OF METABOLITES IN CEREBROSPINAL-FLUID COMPARED WITH PLASMA (Abstract Available)
Author(s): TENBRINK HJ; VANDENHEUVEL CMM; POLLTHE BT; WANDERS RJA; JAKOBS C
Corporate Source: FREE UNIV AMSTERDAM HOSP,DEPT PEDIAT,POB 7057/1007 MB AMSTERDAM//NETHERLANDS/; WILHELMINA CHILDRENS HOSP/UTRECHT//NETHERLANDS/; UNIV AMSTERDAM,ACAD MED CTR/1105 AZ AMSTERDAM//NETHERLANDS/
Journal: JOURNAL OF INHERITED METABOLIC DISEASE, 1993, V16, N3, P587-590

ISSN: 0141-8955
Language: ENGLISH Document Type: ARTICLE
Abstract: **Peroxisomes** are the metabolic sites of a variety of substrates that are poorly oxidized in mitochondria. Impairment of one or more **peroxisomal** functions results in serious disease. Most of the **peroxisomal** disorders present with severe neurological abnormalities like psychomotor retardation, hypotonia, seizures, hearing deficiencies and ocular involvement (see Wanders et al (1988) for a review). In plasma from patients with **peroxisomal** disorders very long-chain fatty acids (VLCFA), pipecolic acid (PIPA), bile acids, and pristanic (PrA) and phytanic (**PhA**) acids accumulate in varying degree.

Little is known about the pathogenesis of neurological symptoms encountered in **peroxisomal** disorders. If disturbances in metabolism of **peroxisomal** substrates affect the central nervous system function, levels of these substrates in cerebrospinal fluid (CSF) may be of special relevance. So far, only PIPA concentrations in CSF have been determined (Kok et al 1987). In this paper we report the analysis of VLCFA, bile acids di- and trihydroxycholestanoic acids (DHCA and THCA), PrA and **PhA** in CSF from healthy individuals and some patients with various **peroxisomal** disorders, and comparison is made with plasma levels.

10/3,AB/8 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02140759 Genuine Article#: KE603 Number of References: 25
Title: HETERONUCLEAR NMR ANALYSIS OF UNSATURATED FATTY-ACIDS IN POLY(3-HYDROXYALKANOATES) - STUDY OF BETA-OXIDATION IN PSEUDOMONAS-PUTIDA (Abstract Available)
Author(s): DEWAARD P; VANDERWAL H; HUIJBERTS GNM; EGGINK G
Corporate Source: AGROTECHNOL RES INST,POB 17/6700 AA WAGENINGEN//NETHERLANDS/; AGROTECHNOL RES INST,POB 17/6700 AA WAGENINGEN//NETHERLANDS/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1993, V268, N1 (JAN 5), P315-319
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: Poly(3-hydroxyalkanoates) (PHAs) were isolated from Pseudomonas putida KT2442 cultivated on petroselenic acid, oleic acid, and linoleic acid to study beta-oxidation of unsaturated fatty acids. Both saturated and unsaturated medium chain length 3-hydroxy fatty acids were found to be constituents of these polymers. With the aid of proton-detected multiple quantum coherence and proton-detected multiple bond coherence NMR spectra the structures of the unsaturated monomers were identified as 3-hydroxy-5-cis-tetradecanoate for **PHA** produced on oleic acid, and 3-hydroxy-6-cis-dodecanoate and 3-hydroxy-5-cis-8-cis-tetradecadienoate for **PHA** produced on linoleic acid. The identified structures, which are derived from fatty acid degradation intermediates, indicate a degradation of oleic acid via the enoyl-CoA isomerase-dependent route and a degradation of linoleic acid via the dienoyl-CoA reductase-dependent route.

10/3,AB/9 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01264453 Genuine Article#: GK061 Number of References: 32
Title: LOOSENING OF CELL-CYCLE CONTROLS OF HUMAN-LYMPHOCYTES UNDER THE ACTION OF TUMOR PROMOTER TPA (Abstract Available)
Author(s): VINOGRADOV AE; EZHEVSKY SA; ROSANOV JM; KAZHDAN IA; ZWEIBACH AS
Corporate Source: ACAD SCI USSR,INST CYTOL,TIKHORETSKYH AVE 4/ST PETERSBURG 194064//USSR/; MED INST 1/ST PETERSBURG//USSR/

Journal: CELL PROLIFERATION, 1991, V24, N5, P493-505
Language: ENGLISH Document Type: ARTICLE
Abstract: The effect of tumour promoter TPA (12-O-tetradecanoylphorbol-13-acetate) on the cell cycle of human peripheral blood lymphocytes stimulated by phytohaemagglutinin (**PHA**) in vitro was studied and it was found that TPA caused cells to accumulate in all the cell cycle phases. This accumulation took place preferentially at later culture passages, when lymphocytes stimulated by **PHA** alone stopped mainly in G0/G1 phases. Other effects of TPA were cell induction to enter higher DNA ploidy and to survive and even synthesize DNA under colchicine block of mitosis or under cytochalasin block of cytokinesis. In addition, in experiments in which a transitory block through the G1 phase of cell cycle was applied with use of aminopterin, we could show that a fraction of TPA-treated cells still entered the active phase of DNA synthesis. These findings suggest that TPA causes cell cycle controls to become loose, thereby enhancing adaptability of human lymphocytes to various hindrances in the course of cell cycle and eventually causing them to acquire characteristics known to be common for tumour cells.

10/3,AB/10 (Item 1 from file: 94)
DIALOG(R)File 94:JICST-EPlus

00433115 JICST ACCESSION NUMBER: 87A0307095 FILE SEGMENT: JICST-E
Role of acidic phospholipids in tissue distribution of quinidine in rats.
NISHIURA A (1); MURAKAMI T (1); HIGASHI Y (1); YATA N (1)
(1) Hiroshima Univ., Hiroshima, JPN
J Pharmacobio Dyn, 1987, VOL.10,NO.3, PAGE.142-147, FIG.1, TBL.3, REF.22
JOURNAL NUMBER: S0989AAS ISSN NO: 0386-846X CODEN: JOPHD
UNIVERSAL DECIMAL CLASSIFICATION: 615.272.4
LANGUAGE: English COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Journal
ARTICLE TYPE: Original paper
MEDIA TYPE: Printed Publication
ABSTRACT: The mechanism of interorgan variation in tissue distribution of quinidine was investigated from a viewpoint of binding characteristics to phospholipids and the composition of phospholipids in various tissues. The order of binding of quinidine to an individual standard phospholipid, expressed as a product of the association constant (K) and the number of binding sites (n), was : phosphatidyl ethanolamine (PhE)<dipalmitoyl phosphatidyl choline (saturated PhC).LEQ.phosphatidyl choline (unsaturated PhC)<phosphatidyl inositol (PhI)<phosphatidyl glycerol (PhG)<phosphatidic acid (**PhA**)<phosphatidyl serine (PhS). Thus, quinidine was found to bind preferentially to acid phospholipids such as PhS, **PhA**, PhG, and PhI. The greatest binding was obtained in PhS among the various phospholipids and was more than 300-fold that of neutral phospholipids such as PhC and PhE. The concentration of individual components of phospholipids in the lung, kidney, liver and heart was determined using a two dimensional thin-layer chromatography. The concentration of PhS, highly responsible for the quinidine binding to phospholipids in each tissue, was ranked in the following order : heart<liver<kidney<lung. The contribution of PhS to quinidine binding was more than 86% in all tissues. A good correlation between the concentration of PhS in each tissue and the Ct/Cp ratio in vivo was obtained (r=0.984). Thus, it was concluded that the tissue distribution of quinidine in vivo depended on the composition of phospholipids in tissues and that a determinant of interorgan variation in the tissue distribution of quinidine was the concentration of PhS in the tissues.(author abst.)

10/3,AB/11 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

12361630 PASCAL No.: 96-0006371
Negative selection in hepatic tumor promotion in relation to cancer risk assessment
ANDERSEN M E; MILLS J J; JIRTLE R L; GREENLEE W F
SETZER R Woodrow JR, ed; GRAY L Earl JR, ed
ICF Kaiser Engineers, Morrisville NC 27560, USA
US environmental protection agency, national health environmental effects res. lab., Research Triangle Park NC 27711, USA
US Environmental Protection Agency. Health Effects Research Laboratory, Research Triangle Park NC, USA.
Biological mechanisms and quantitative risk assessment. Symposium (USA) 1993-11-01
Journal: Toxicology : (Amsterdam), 1995, 102 (1-2) 223-237
Language: English
Mechanistic studies with phenobarbital (PB), 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD) and other liver tumor promoters support a general model of promotion involving negative selection where specifically-mutated cells derive a growth advantage in the presence of persistent mitosuppression. Exposure to these liver tumor promoters appears to transiently enhance hepatocyte replication, presumably via transcriptional activation of growth regulatory genes, leading to a homeostatic increase in mitoinhibitory growth factors in the liver to constrain proliferation. Transforming growth factor beta 1 (TGF- beta), a potent mitoinhibitory growth factor for hepatocytes, has been associated with the mitosuppression caused by PB and certain **peroxisomal** proliferators. Escape from TGF- beta mitosuppression may involve loss or alteration of function of the mannose 6-phosphate/insulin-like growth factor II (M6P/IGFII) receptor, which is required for TGF- beta I activation, or alterations of the TGF- beta types I, II and III signal transduction receptors. A risk assessment based on a negative selection mechanism could be conducted for tumor promotion endpoints with TCDD and compared with current approaches that implicitly regard TCDD as an initiator. Benchmark dose calculation using centrilobular induction of cytochromes P450 1A1 and 1A2 as a surrogate for periportal growth stimulation would provide a rational starting point for application of conventional safety factor approaches, similar to those used with non-cancer effects. In the future, tissue and plasma concentrations of specific growth factors, e.g. TGF- beta or hepatocyte growth factor, HGF, might be considered as more direct dose surrogates for tumor-promoting effects of xenobiotics. Uncertainty factor adjustments to a TCDD benchmark dose calculation should eventually rely on direct knowledge of regulation of specific growth regulatory genes and their receptors in relevant species and on species differences in TCDD **pha**

10/3,AB/12 (Item 2 from file: 144)
DIALOG(R)File 144:Pascal

11063032 PASCAL No.: 93-0570041
Peroxisomal disorders : concentrations of metabolites in cerebrospinal fluid compared with plasma
'Inherited metabolic disease and the brain'
TEN BRINK H J; VAN DEN HEUVEL C M M; POLL-THE B T; WANDERS R J A; JAKOBS C
Free univ. hosp., dep. pediatrics, 1007 MB Amsterdam, Netherlands
Journal: Journal of inherited metabolic disease, 1993, 16 (3) 587-590
Language: English
Peroxisomes are the metabolic sites of a variety of substrates that are poorly oxidized in mitochondria. Impairment of one or more **peroxisomal** functions results in serious disease. Most of the **peroxisomal** disorders present with severe neurological abnormalities like psychomotor retardation, hypotonia, seizures, hearing deficiencies and ocular involvement (see Wanders et al (1988) for a review). In plasma from

preadi pocytes, while t[redacted]t of C/EBPbeta was not. Transie[redacted] transfection to increase C/ EBPalpha ex[redacted]ession in epididymal preadipoc[redacted]s enhanced their ability to differ entiate. This implicates events upstream of C/EBPalpha but downstream of C/EBPb eta during differentiation as the cause of interdepot variation in preadipocyte differentiation. PPARgamma is expressed after C/EBPbeta and promotes C/EBPalpha expression. Thiazolidinediones, which act by binding to PPARgamma are less effe ctive in inducing epididymal than perirenal preadipocyte differentiation. TNFal **pha** blunts C/EBPalpha expression and blocks effects of PPARgamma. We found that epididymal preadipocytes release more TNFalpha than perirenal cells, and that e xposing differentiating perirenal cells to concentrations equivalent to those in epididymal cultures blunted their differentiation. Based on our preliminary fi ndings, our hypothesis is that regional variation in TNFalpha release and PPARga mma activity plays a central role in effects of fat depot origin on ability of p readipocytes to express C/EBPalpha and differentiate. We propose the following specific aims to test this hypothesis. Aim 1 is to establish the role of TNFalp ha in regional variation of preadipocyte differentiation and C/EBPalpha expressi on. Aim 2 is to determine the role of PPARgamma and related factors in causing variation among depots in preadipocyte differentiation, C/EBPalpha expression, a nd TNFalpha release. Aim 3 is to assess the relevance of our findings to humans by determining if preadipocyte C/EBPalpha and PPARgamma expression and TNFalpha production differ between human omental and abdominal subcutaneous preadipocytes from the same subjects. The studies we propose to understand the mechanism(s) underlying interdepot variation may add not only to knowledge about effects of t issue site on the capacity of progenitors within that tissue to differentiate, b ut also about why fat tissue mass, metabolism, hormone responsiveness, and funct ion vary among depots. These studies will point to potential future strategies for altering function in specific fat depots.

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Set     Items   Description
S1       1868   POLYHYDROXYALKANOATE?
S2         29   S1 AND PEROXISOME?
S3         11   RD (unique items)
S4         36   S1 AND FUSION?
S5         16   RD (unique items)
S6        371   PEROXISOM? AND TARGET? AND FUSION?
S7          0   S6 AND PHA
S8         31   PHA AND PEROXISOM?
S9         23   S8 NOT S3
S10        14   RD (unique items)
? s s6 and protein?

Processing
Processed  10 of  20 files ...
Completed processing all files
             371  S6
         6156631  PROTEIN?
     S11     366  S6 AND PROTEIN?
? s s11 and express? and plant?

Processing
Processed  10 of  20 files ...
Processing
Completed processing all files
             366  S11
         3529239  EXPRESS?
         7798608  PLANT?
     S12      61  S11 AND EXPRESS? AND PLANT?
? rd

>>>Duplicate detection is not supported for File 306.
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patients with **peroxis** disorders very long-chain tty acids (VLCFA), pipecolic ac (PIPA), bile acids, and p anic (PrA) and phytanic (**PhA**) acids accumulate in varying degree. Little is known about the pathogenesis of neurological symptoms encountered in **peroxisomal** disorders

10/3,AB/13 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00319861
IDENTIFYING NO.: 1F32HL10410-01 AGENCY CODE: CRISP
PGC-1: REGULATOR OF MITOCHONDRIAL METABOLISM IN HEART
PRINCIPAL INVESTIGATOR: HUSS, JANICE M
ADDRESS: WASHINGTON UNIV SCHOOL OF MED 660 SOUTH EUCLID AVENUE ST LOUIS, MO 63110-1093
PERFORMING ORG.: WASHINGTON UNIVERSITY, ST. LOUIS, MISSOURI
SPONSORING ORG.: NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
FY : 2001
SUMMARY: The adult mammalian heart utilizes fatty acids as its predominant energy source. In hypertrophy, the heart switches from fatty acid oxidation (FAO) to glucose m etabolism for energy generation. The **peroxisome** proliferator-activated receptor alpha (PPARalpha) regulates expression of genes encoding key FAO enzymes, FFARal **pha** is a nuclear receptor transcription factor that functions with co-activator proteins to activate transcription. The recently identified co-activator, PPAR g amma co- activator-1 (PGC-1), is a candidate for cooperating with PPARalpha to r egulate FAO enzyme genes. Both proteins are down-regulated during cardiac hypert rophy, implicating a role for PPARalpha/PGC-1 signaling in the hypertrophy gene program. PGC-1 has also been shown to regulate genes involved in mitochondrial b iogenesis by a mechanism independent of its function with PPARalpha. The specifi c aims are to 1) characterize the role of PPARalpha and its, co-activator, PGC-1 , in the control of cardiac mitochondrial energy transduction pathways, 2) co-ac tivator, PGC-1, in the control of cardiac mitochondrial energy transduction path ways, 3) map functional domains within the PGC-1 molecular relevant to the devel opment of dominant inhibitory mutants, and 3) determine whether PPARalpha/PGC-1 signaling plays a primary role in the cardiac myocyte hypertrophy program. Long- term goals involve development of genetically engineered animals to investigate the roles of PGC-1 in regulating cardiac metabolism in vivo.

10/3,AB/14 (Item 2 from file: 266)
DIALOG(R)File 266:FEDRIP

00305921
IDENTIFYING NO.: 5R01DK56891-02 AGENCY CODE: CRISP
EFFECT OF FAT DEPOT ORIGIN ON PREADIPOCYTE FUNCTION
PRINCIPAL INVESTIGATOR: KIRKLAND, JAMES L
ADDRESS: BOSTON MEDICAL CENTER 88 E NEWTON ST (F4) BOSTON, MA 02118
PERFORMING ORG.: BOSTON MEDICAL CENTER, BOSTON, MASSACHUSETTS
SPONSORING ORG.: NAT INST OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
FY : 2001
SUMMARY: Despite similar nutrient and hormone exposure, there is wide variation in lipid content and function of different fat depots within the same individual. The fa t depot from which preadipocytes are cultured affects their capacity to differen tiate, even in cells cultured for several cell generations and studied at the si ngle cell level. Thus, mechanisms intrinsic to adipose cells contribute to inte rdepot variation in fat tissue function, in addition to variation among depots d ue to extrinsic factors, such as vascular supply and innervation. We found that expression of C/EBPalpha was blunted during differentiation in epdidymal

gene promoter (UPR-ICL), high intracellular NADP-IDH activity was observed. Comparison of amino acid sequences and phylogenetic tree analysis with NADP-IDH enzymes from all reported eukaryotic sources revealed that mammalian mitochondrial NADP-IDHs formed a cluster, as did plant NADP-IDHs. CtIdp2p and other yeast NADP-IDHs were not included in these clusters and seemed to diverge at an early stage from all other enzymes of higher eukaryotes. Ps-NADP-IDH had no typical C-terminal **peroxisomal targeting** signal and no processing was demonstrated at the N-terminus. However, we could find a region near the N-terminus of the protein with high similarity to both the putative N-terminal **peroxisomal targeting** signal sequence of Fox3p of S. cerevisiae and an internal region of Pox4p of C. tropicalis. The results of northern blot analysis indicated that the biosynthesis of CtIdp2p was induced in a medium containing alkanes as a carbon source, where profuse proliferation of **peroxisomes** is observed.

9/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09570195 97433282 PMID: 9288922
Cytosolic aspartate aminotransferase encoded by the AAT2 gene is **targeted** to the **peroxisomes** in oleate-grown **Saccharomyces** cerevisiae.
Verleur N; Elgersma Y; Van Roermund CW; Tabak HF; Wanders RJ
Department of Clinical Biochemistry, Academic Medical Center, Amsterdam, The Netherlands.
European journal of biochemistry (GERMANY) Aug 1 **1997**, 247 (3) p972-80, ISSN 0014-2956 Journal Code: EMZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Fatty acid beta-oxidation in **peroxisomes** requires the continued uptake of fatty acids or their derivatives into **peroxisomes** and export of beta-oxidation products plus oxidation of NADH to NAD. In an earlier study we provided evidence for the existence of an NAD(H) redox shuttle in which **peroxisomal** malate dehydrogenase plays a pivotal role. In analogy to the NAD(H)-redox-shuttle systems in mitochondria we have investigated whether a malate/aspartate shuttle is operative in **peroxisomes**. The results described in this paper show that **peroxisomes** of oleate-grown **Saccharomyces** cerevisiae contain aspartate aminotransferase (AAT) activity. Whereas virtually all cellular AAT activity was **peroxisomal** in oleate-grown cells, we found that in glucose-grown cells most of the AAT activity resided in the cytosol. We demonstrate that the gene AAT2 codes for the cytosolic and **peroxisomal** AAT activities. Disruption of the AAT2 gene did not affect growth on oleate. Furthermore beta-oxidation of palmitate was normal. These results indicate that AAT2 is not essential for the **peroxisomal** NAD(H) redox shuttle.

9/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09517789 96421645 PMID: 8824293
Analysis of the carboxyl-terminal **peroxisomal targeting** signal 1 in a homologous context in **Saccharomyces** cerevisiae.
Elgersma Y; Vos A; van den Berg M; van Roermund CW; van der Sluijs P; Distel B; Tabak HF
Department of Biochemistry, Academic Medical Centre, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands.
Journal of biological chemistry (UNITED STATES) Oct 18 **1996**, 271 (42) p26375-82, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: Journal Article